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OSTEITIS FIBROSA *

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In 1891 von Recklinghausen described a complexity of changes in different parts of the bony skeleton. Such changes consisted in a transformation of the bone marrow into a fibrous connective tissue rich in giant cells, and a resorption of the compact cortex followed by a replacement with finely porous and often uncalcified new bone. Within these fibrous areas were cysts, which von Recklinghausen related to liquefaction — necrosis of the connective tissue. Here, also, he found the so-called solid brown tumors composed of numerous, multinuclear giant cells in a spindle cell matrix containing deposits of hemosiderin. These tumors were described as true giant cell sarcoma, or a tumor forming osteitis fibrosa, or as osteitis fibrosa with cysts and brown tumors.

In addition to this generalized osteitis fibrosa a localized type of the disease was described. Bones, otherwise uninvolved, contained localized areas of osteitis fibrosa with cysts and giant cells. These cases were accordingly treated as true sarcoma.

The discussion of the cause and origin of the cysts and giant cell tumors has produced a large and rich literature. Without exception until 1907 these brown or giant cell tumors were considered to be malignant. In this year Lubarsch, studying the case of Gaugele, brought forward proof that the giant cell tumor was an entirely benign formation of regenerative and resorptive character. Konjetzny was led to the same conclusion by his studies of the disease. Bloodgood considered the process a benign neoplasm and as early as 1910 advocated conservative treatment.

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Different theories were advanced from time to time to explain the origin of the cysts in the generalized form of the disease, as well as the single solitary cysts. Beneke suggested that trauma was the basis for the formation of the solitary bone cysts and compared them to the apoplectic cysts of the brain. The correctness of this view was proved by my preceptor Pommer, in 1919, through the study of a case of cysts of the humerus in a 22 year old woman. From the study of the cyst contents and of the tissue surrounding the cyst Pommer established the direct proof for the hematomatous character of the process. The contents of the cyst, the hemosiderin deposits in its wall and in the bone marrow spaces of the surrounding bone, and furthermore the calcification of the fibrin deposits permit of no other explanation. The serous and albuminous contents of the solitary cysts are a mixture of the partly resorbed hemorrhages and new transudates. The pressure of hemorrhages upon the veins produces a stasis of tissue fluids, which causes the liquefaction of the blood content of many of the cysts, and edema of the tissues in the immediate vicinity. The blood vessels enclosed in the rigid compact bone have only a limited possibility of relieving disturbances of circulation, in contrast to the vessels in more loosely constructed organs.

The localized areas of loose fibrous tissue replacing the bone marrow were regarded by Pommer as effects of the congestion and irritation produced by the hemorrhages. These changes, designated as "phlegmasia," the result of a combination of congestion and reactive, irritative and inflammatory processes, are therefore secondary. As a result of functional mechanical factors and decreased fluid pressure a richly vascular and delicate network of new bone is often formed. This new bone is not a primary change, but, like the phlegmasia, a secondary result. The more active resorption of the surrounding bone is also a secondary result; it is dependent upon the pressure of hemorrhages and the increased transudation. These two factors, pressure from hemorrhages and increased transudation, produce increased bone resorption and eccentric atrophy of the bone and afford, also, the possibility for secondary hemorrhages with progressive cyst formation.

Following these fundamental observations by Pommer, Looser, in 1924, investigated the nature of the cysts in the so-called generalized osteitis fibrosa. Looser came to the conclusion that the multiple

cysts of this disease also had their origin in the hemorrhages into bone marrow following trauma.

In spite of the great frequency of trauma in childhood, progressive cysts are found in relatively few cases. The site of the trauma is a decisive factor. Cyst formation does not follow injuries to the shaft of the bones as frequently as injuries to the epiphyses. The explanation lies in the fact that the richly vascular and porous juvenile epiphysis is especially prone to oft repeated hemorrhages subsequent to a comparatively slight trauma. It is also of great importance that the products of trauma — hemorrhage, edema and fluid — remain localized. Only in incomplete or complete fractures without tearing of the periosteum are the preliminary conditions present for the formation of cysts. Under these circumstances a primary hemorrhage leads to pressure upon the efferent vessels. Finally the maintenance of functional activity, either partial or total, acts as a pathological irritant, important for the production of secondary hemorrhages with the above mentioned sequellae. Whenever the trauma tears the periosteum, even in incomplete fractures, the blood escapes into the surrounding soft tissues and cyst formation does not follow.

The interpretation by Lubarsch of the so-called giant cell tumor as a regenerative and resorptive process, and the recognition by Pommer of the blood cysts as a progressive hematoma formation clarified the developmental picture of osteitis fibrosa. Thus the two processes, which were considered characteristic of the so-called generalized osteitis fibrosa, were shown to be dependent on localized conditions. Furthermore, after this knowledge of the origin of the "phlegmasia" changes in the bone marrow, there no longer remained the necessity of explaining the development of the loose, fibrous transformations of the bone marrow as an independent form of osteitis fibrosa.

Yet, almost everywhere, osteitis fibrosa was considered as an independent disease and its origin was ascribed to various causes. Inflammatory processes especially were considered responsible for the localized type of the disease. Other observers believed the localized as well as the generalized form of osteitis fibrosa to be a disease of unknown origin. Von Recklinghausen advanced the conception that generalized osteitis fibrosa was definitely related to osteomalacia. In some instances the osteofibrotic changes are associated with hyperplasia or tumors of the parathyreoid gland, and in these cases

it is possible to find the productive mechanism. This finding points to a relation between generalized osteitis fibrosa and osteomalacia. In the genesis of osteomalacia, disturbances of the calcium metabolism are responsible, and the parathyreoid gland is closely associated with such disturbances.

Such cases of osteitis fibrosa, associated with hyperplasia or tumors of the parathyreoid gland, do not contradict the conception which regards osteitis fibrosa as a secondary process dependent upon circulatory disturbances. In these cases the circulatory disturbances follow the pathological action of functional trauma upon the softened bone.

Regarding the relation between the parathyreoid gland and calcium metabolism, it is important to note that according to Biedl the hormone not only influences the secretion of calcium but also favors its resorption. As the calcium content of the blood rises, the elimination of calcium by the stomach decreases. The hormone possesses the property of fixing the calcium in the blood and closing its barriers of escape into the tissues. Hypersecretion by the parathyreoid gland leads to osteitis fibrosa, in which condition the blood calcium content is substantially increased. It is noteworthy that hyperplasia or tumors of the parathyreoid gland are frequently observed in osteomalacia and rickets.

The relation between parathyreoid hypersecretion and osteitis fibrosa is demonstrated by the recent researches of Jaffe, Bodansky and Blair. Through the administration of parathyreoid extract — for example parathormon — these investigators were able to influence calcium metabolism and produce osteitis fibrosa.

As already mentioned, Pommer traced the fibrous transformation of the bone marrow to localized congestion and irritative influences. Later, while studying a case of osteomalacia, he had a glimpse of the relation between osteitis fibrosa and osteomalacia. He was furthermore able to prove the dependence of osteitis fibrosa upon osteomalacia, and especially its dependence upon the mechanical effects of functional activity.

Following these observations, I made extensive studies (1925) to explain the genetic relation between osteomalacia, rickets and osteitis fibrosa. I found that the parts of the skeleton that are affected especially by mechanical function and strain undergo bending and cracking because of the insufficient calcification. Under

these conditions, due to the peculiar structure and circulatory system of the bone, a permanent congestion of the blood and lymph vessels is produced. This congestion, together with the continuous mechanical irritation, leads to osteitis fibrosa. The fibrosis of the bone marrow and the increased rebuilding of bone are, therefore, not independent processes; they are secondary results of the localized circulatory disturbances which follow the action of functional, mechanical trauma upon insufficiently calcified and softened bone.

This year (1931) I confirmed these conclusions by experimental and clinical studies of rickets and scurvy, and by further studies of osteomalacia. In all these diseases I found regularly osteitis fibrosa (Figs. 1 to 8).

The development of osteitis fibrosa following a trauma, producing a congestion, a resorptive inflammation and finally a progressive hematoma, is comparable to the induration in parenchymatous organs following chronic passive congestion. Osteofibrotic changes are also observed in inflammatory processes as a result of the congestion. An example of this is to be found in odontogenetic osteitis fibrosa of the jaw. Similar changes exist in the immediate vicinity of metastases to bones, as Wagoner has pointed out, in callus formations and pseudo-arthrosis, in gout, and about tubercular and syphilitic bone lesions. By these processes are produced the same pressure and congestion influences as occur after traumatic hemorrhage into the bone marrow.

This large and varied material establishes beyond doubt the secondary nature of osteitis fibrosa and disproves the theory that it is two independent processes.

To close with a few general considerations: von Recklinghausen advanced the opinion that, in the bony system, the secondary symptoms and sequellae control the disease picture and mask the basic factors. This important conception explains the great difficulty in arriving at a definite conclusion from the final picture of the disease process. We have seen that in rickets, scurvy and osteomalacia, the disease picture is dominated by osteitis fibrosa. It is only by exacting histological methods, designed to show both the calcified and non-calcified bone, and by the study of many large sections from various bones, seeking the primary changes, unobscured by osteitis fibrosa, that we were able to establish the basic nature of the disease. Without such careful methods, an incorrect diagnosis of primary

osteitis fibrosa will be made, thus confirming the current misconceptions on this subject.

Of all the conditions that are responsible for the origin of osteitis fibrosa, the most decisive are the peculiar structure and the circulatory system of bone which allow only a slight possibility for compensatory adaption to circulatory disturbances. Furthermore, use of the bone acts as an irritant under the given pathological conditions.

The functional viewpoint has proved to be of the greatest importance in explaining the question of the relation between osteitis fibrosa, osteomalacia and rickets. Likewise in general pathology the application of the functional viewpoint promises to clarify the origin and relationship of many obscure pathological changes.

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DESCRIPTION OF PLATES

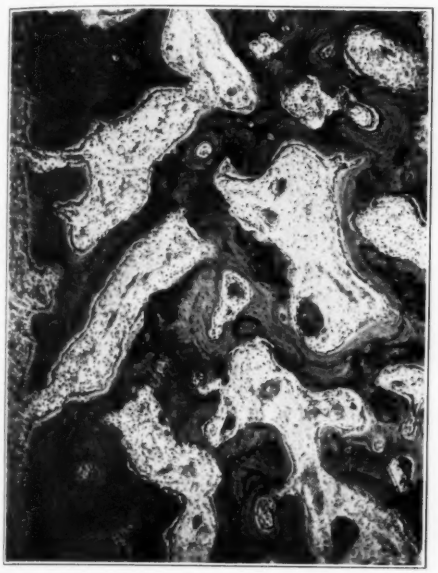
PLATE 40

FIG. 1. Osteitis fibrosa of the alveolar bone of the lower jaw of a dog in experimental rickets, with rebuilding of the bone and fibrosis of the bone marrow. Hyperemia of the blood vessels is shown.

FIG. 2. Microscopic section of the humerus of a 2 year old girl with rickets.

FIG. 3. Osteitis fibrosa in rickets, showing deeply stained (calcified) bone surrounded by a newly formed, non-calcified bone. Hyperemia of the fibrotic bone marrow is also shown. High power picture of Fig. 2.

FIG. 4. Femur of a 3 year old girl with rickets, showing new formation of uncalcified bone and fibrosis of the bone marrow.



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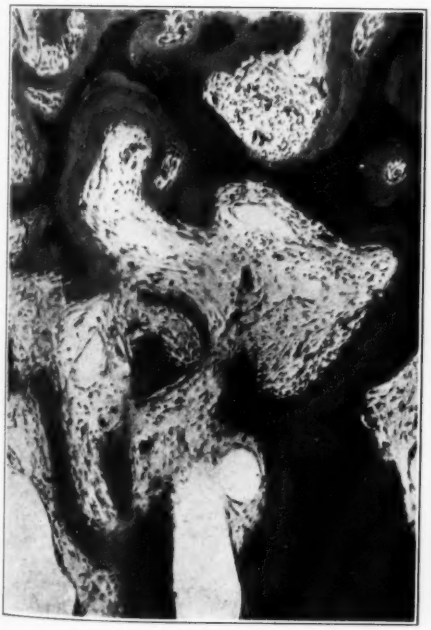

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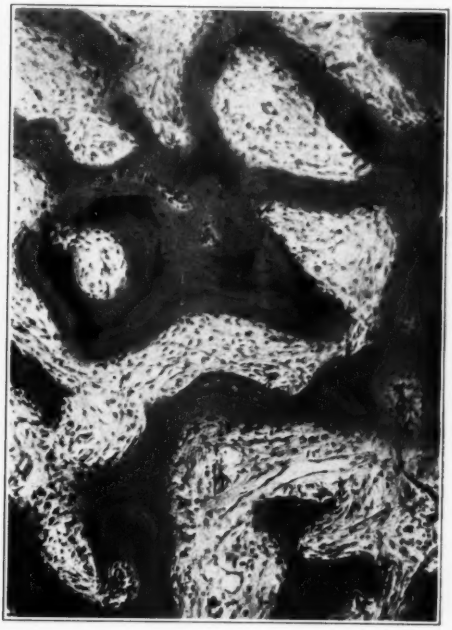

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Osteitis Fibrosa

Plate 41

Fig. 5. Fibrosis of the bone marrow and rebuilding of the bone in rickets in a 10 months' old boy. On the left side of the figure lymphoid bone marrow is seen.

Fig. 6. Tibia, showing "phlegmasia" of the bone marrow and increased rebuilding of the bone in a case of osteomalacia in a 48 year old man.

Fig. 7. Fibrosis of the bone marrow and new formation of uncalcified bone in osteomalacia in a 78 year old woman. Calcified bone deeply stained.

Fig. 8. Osteitis fibrosa with fibrosis of the bone marrow and rebuilding of the bone in osteomalacia in an 80 year old woman.

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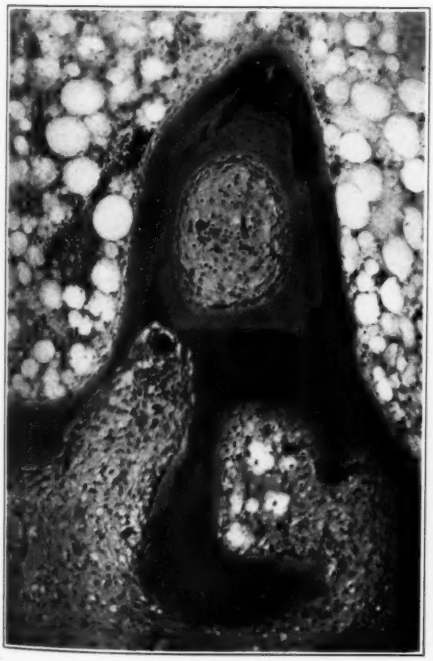

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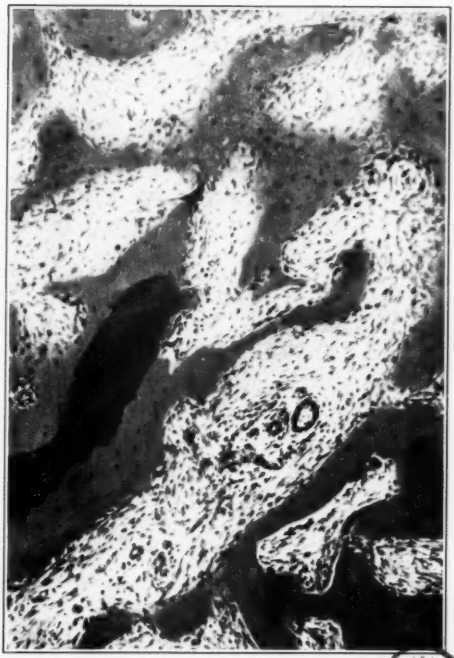

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Lang Osteitis Fibrosa

VACCINAL INFECTION OF THE CHORIO-ALLANTOIC MEMBRANE OF THE CHICK EMBRYO *

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Notwithstanding a certain amount of confusion which has at times existed concerning the possibility of an identity of fowl-pox and variola-vaccinia, there are now several studies on record which show that although chickens are susceptible to infection with vaccine, fowl-pox is a distinct disease both immunologically and in the cytology of its lesions (Levaditi and Nicolau, Ledingham, Lowenthal *et als.*, Andervont, Woodruff). Andervont [1] was able to demonstrate Guarnieri bodies in vaccinal lesions of chickens, and this was confirmed by Woodruff,[2] who pointed out essential distinctions between these inclusions and the Bollinger bodies of fowl-pox.

In a recent communication we reported the successful infection of the chorio-allantoic membrane of chick embryos with vaccinia virus, following the method of inoculation described by Woodruff and Goodpasture [3] in their study of fowl-pox of this membrane.[4] In the present report we wish to describe in greater detail vaccinal infection of the membranes of the chick embryo, with especial reference to the cytology of the lesion and the seeming relationship between the Guarnieri bodies and the Paschen corpuscles which constitute the specific elements.

While the Guarnieri bodies are by every investigator of the subject regarded as specific for variola-vaccinia, there is perhaps still skepticism as to the specificity of the Paschen granules and no one as yet has been able to demonstrate a relationship between these two important structures, although many agree with the view of Paschen that the elementary corpuscles represent the actual virus of the disease.[5]

Vaccinal lesions in the chorio-allantoic membrane of embryo chicks afford an unusual opportunity to study both of these morphological elements and, we believe, furnish good evidence that the

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Guarnieri bodies represent, in part at least, colonies or masses of Paschen corpuscles.

Technique

Bacteria-free virus in fresh infected rabbit's testis (Levaditi neurovaccine) was used to initiate the infection. While we have been able to infect the chick membrane with glycerinated virus, the result is more uncertain, and we recommend the use of fresh infected tissue from the rabbit's testis as a constant source of original virus.

Since the infection "takes" rapidly and the lesion evolves readily, requiring only two or three days for its development, we have found it most satisfactory to use chick embryos of 12 days' incubation. At this stage the membrane is well formed and easily accessible. By candling the egg the air-cell and the membrane can be outlined with a wax pencil. A thin coat of melted paraffine is laid over the shell where the window is to be cut. The egg is then placed in a bowl of warm water (40° C) and rested upon a mass of plasticene which has been properly indented to receive it. This holds the egg in the suitable position. The water should come almost up to but not over the paraffined surface. With a hard steel trocar, ground with a triangular end to a sharp point, a window 1 or 1½ cm. square is cut in the paraffined surface. If the sides have been well cut the overlying shell can be easily removed by lifting it at one corner with fine pointed forceps. The exposed shell membrane may be unruptured, though frequently it is more or less injured. A thin coat of melted paraffine (about 45° C) is laid over the cut edges of the shell and the exposed shell membrane with a cotton swab. With the point of a pair of fine curved forceps the shell membrane may be torn from beneath outward on three sides, folded outward and the fourth side cut with small scissors. These procedures tend to prevent infection from broken bits of shell. The instruments used are kept sterile by passing them through the flame of a Bunsen burner.

When the membrane is exposed a bit of infected tissue (rabbit testis or membrane) about the size of a pin-head is placed upon it. A mixture of sterile vaseline and paraffine in a 10 cc. record syringe is used to lay a ring about the opening and upon this a sterile cover glass is placed and pushed down to seal it completely. The egg is then returned to the incubator with the window up. It may be ex-

amined on succeeding days by placing the window under a dissecting microscope.

When it is desired to open the egg to examine and remove the infected area, the cover slip is pulled off and frequently the vaseline comes off with it. If not, it can easily be scraped off with a sterile scalpel. The window is enlarged to the desired size by breaking off the edges with sterile forceps. The infected membrane may then be cut out with small scissors and placed in a petri dish containing sterile isotonic fluid.

The Vaccinal Lesions of the Membrane

After 24 hours the membrane appears somewhat thicker, grayer and more opaque about the bit of inoculum, and after 48 hours there is a zone about 1 cm. wide, thickened, gray, opaque and usually flecked with small hemorrhages. Sometimes almost the entire area is red and hemorrhagic. At this stage there is a gray advancing margin which is best for histological study. In the center there may be a brownish area of necrosis, variable in size. After the lesion has been extirpated a bit of tissue from the thickened and hemorrhagic area is removed with scissors and smears from this are stained by Morosow's method [6] to determine the presence of Paschen bodies. These bodies appear in enormous numbers in the infected membranes at the 48 hour period and after. Their presence is diagnostic of vaccinal infection. Other smears are stained with Loeffler's methylene blue to determine the presence of bacteria. If Paschen corpuscles are abundant and no bacteria are demonstrable, a piece of infected membrane about 0.5 mm. in diameter is inoculated upon the membrane of each of five or six embryos for continuing the passage. Pieces of the remaining infected membrane are then inoculated into culture media and others are kept in the icebox or in glycerol (50 per cent in 0.9 per cent saline). Tissue for histological study is taken from the advancing margin and fixed immediately.

The preparations used in the present histological study were fixed in Zenker's solution (10 per cent glacial acetic acid), and stained in a 2 per cent aqueous solution of acid fuchsin for 10 to 30 minutes, washed and counterstained about 30 seconds with Loeffler's methylene blue, differentiated in absolute alcohol, cleared in xylol and mounted in cedar oil.

Infected chick embryos usually die on the fourth day after inoculation, and we have found the 48 hour period best for transplantation and general study, though 72 hours is often a satisfactory interval.

Serial Transfers

In one series of experiments the virus was carried through eight generations in embryos, in another series it reached the fourteenth generation. For some reason, probably technical, the fifteenth generation failed to "take." In these passages there did not appear to be any diminution in virulence, so far as the appearances of the lesions were concerned.

The virus in the eighth generation was inoculated upon the skin of baby chicks after plucking the down. Macroscopic nodules, about 1 mm. in diameter, corresponding to down follicles, appeared at the site of inoculation within three days. Remaining apparently stationary for two or three days they rapidly receded. Two weeks after their recovery from vaccinia these chicks were inoculated with fowl-pox virus, and infection ensued which ran a typical course. This experiment confirms the result of other observers, that vaccinia in the chick confers no immunity to fowl-pox.

Histology

The infected membrane may be considerably thickened, though not so much so as in fowl-pox. The swelling is due to a variety of causes, least of all to cellular hyperplasia, which is the chief response to fowl-pox.

In the vaccinal infection inflammatory changes and hemorrhage are mainly responsible for the increase in thickness, and this is especially marked in the older areas of involvement. At the extreme advancing edge one finds the latest effects which are characterized by moderate edema, perhaps some capillary hemorrhage and slight hyperplasia, both of ectodermal epithelium and endothelial cells. The entodermal epithelium is slightly, if at all, affected. As the earlier infected areas are approached, ectodermal epithelium is found to be necrotic and its capillaries filled with cellular débris. Inflammatory exudate in the mesodermal layer is increased in abundance and consists largely, in addition to red blood cells, of polymorphonuclear leucocytes. There are also admixed with

these, large rounded mononuclear cells which may be either disoriented endothelium or fibroblasts. Occasionally mitotic figures are found in the large cells. The entodermal epithelium in these areas may show considerable hyperplasia, but usually no necrosis. In the earliest areas of infection these changes are accentuated, and there may be inflammatory cells in the entodermal layer and necrosis. At the advancing margin, capillary endothelium sometimes undergoes active focal hyperplasia, indicated by small isolated groups and whorls of these cells in the form of tiny nodules.

From a histological standpoint the virus seems to affect ectodermal epithelium first and most profoundly, mesodermal cells less markedly and entodermal epithelium least of all. No vesicles are formed. The infection seems to spread diffusely and centrifugally, affecting the entire membrane as it goes. There is no evidence thus far that lesions occur in the embryonic tissues other than at and about the site of inoculation in the chorio-allantoic membrane. We have not yet made a study of the dissemination of the virus in the embryo.

Guarnieri Bodies

In sections stained to demonstrate Guarnieri bodies, very marked changes are to be found in practically all types of cells of the fixed tissue. In order to study these changes to the best advantage one must examine the latest stages in the advancing edge of the infection in the membrane. Injury is profound and the cells rapidly undergo disintegration. It seems probable that there is only a short interval in which the cellular inclusions may be seen to best advantage. The Guarnieri bodies develop in abundance in the cells before there is any evidence of inflammatory cellular exudate.

Guarnieri bodies are best observed in the ectodermal cells. Practically all of these cells in the recently infected areas, when intact, show the "included" material in their cytoplasm. Here the bodies occur in irregular clumps and masses, perinuclear or paranuclear in arrangement. The cytoplasmic masses are granular and rather amorphous. The material stains for the most part with fuchsin in our preparations, though sometimes it is flecked with bluish granules. The granular material is fairly dense when properly differentiated and lies in a clear area. The inclusions, when single, are often triangular in shape with the base next to the nucleus. The Guarnieri

material is very abundant and sometimes almost completely replaces the cellular cytoplasm. In other cells it is more dispersed and is scattered through the cytoplasm in fine and coarse granules. Similar intracytoplasmic masses, often reaching a relatively large size, are found abundantly in endothelial cells and fibroblasts of the mesodermal layer. These masses have the morphology and the staining characteristics of Guarnieri bodies. They are especially well marked in the cells composing the endothelial nodules. Isolated fibroblasts show them quite distinctly. They are to be found typically also in endothelial cells lining veins and capillaries. Adventitial cells, as well as endothelial cells, composing the walls of larger blood vessels, often show these characteristic Guarnieri bodies, and one sometimes gets the impression that they are present in smooth muscle cells as well, but this is still doubtful.

The entodermal cells lining the infected area frequently contain numerous Guarnieri bodies, and here they have more the typical structure of these inclusions as they are usually to be seen in the corneal epithelium of the infected rabbit's eye. That is to say, they are apt to be small, single, discrete, more compact and more densely stained. They lie in a clear space next to the nucleus. In areas of hyperplasia of entodermal cells, however, they may become larger and more granular, simulating those of the ectodermal and mesodermal cells.

One gets the impression that the entodermal cells offer considerably more resistance to the infection than the cells of the other two germinal layers, and this may account for the variation in the morphology of the Guarnieri bodies.

It is to be emphasized that the chief, if not all "included," material within any of these cells is that which composes the Guarnieri bodies. This is of great importance in view of the appearance found in smears from the membranes stained by Morosow's method to demonstrate Paschen corpuscles.

Paschen Corpuscles

If fragments of fresh, infected membrane be placed in distilled water and examined under the oil immersion lens, one frequently sees round or oval masses, apparently within the cytoplasm of cells, which are composed almost entirely of minute, uniform granules oscillating rapidly in Brownian motion. These granules have the

size, uniformity of structure and numbers which correspond to the Paschen bodies so abundantly demonstrable in smear preparations. The masses are not so numerous, however, as Guarnieri bodies are known to be in the same tissue, and we have not been able positively to correlate the two structures in fresh preparations.

Smears made directly from a piece of fresh, untreated membrane and stained by the Morosow method show enormous numbers of Paschen corpuscles diffusely scattered, and also in distinct masses.[6] It appears from these smears that the Paschen bodies occur originally in rather sticky groups which, in the process of making the smear, adhere to each other, forming relatively large clumps of material resembling closely similar masses occurring in smears from fowl-pox. About the edges of these clumps are the discrete Paschen bodies which give the clue to the composition of the whole.

In the smears made directly in this way there are a very few intact cells, so that it is difficult usually to detect any relationship between the Paschen corpuscles and cells. However, if a piece of membrane is placed in 1 per cent trypsin solution for 30 minutes, it becomes softened and smears made with it show many intact cells that evidently have become dislodged from the loosened stroma. Many of these cells show in their cytoplasm one or more masses corresponding in size to the Guarnieri bodies, and in those cells which are spread out or partially ruptured by smearing, it can be seen readily that the intracellular structures are composed of compact agglomerations of uniform, round, minute corpuscles, measuring approximately 0.25 microns in diameter, although enlarged by the staining method. These are the Paschen corpuscles, and from a morphological standpoint we have no doubt that the intracellular masses of them are identical with the Guarnieri bodies found so abundantly and so large in the stained sections. No other observed intracellular structures could account for them.

Another experiment indicates that all the dispersed Paschen corpuscles originate from ruptured cells. If one places a small piece of membrane in 1 per cent acetic acid for 5 minutes, then washes it and makes a smear, there are few if any dispersed Paschen corpuscles, but hyaline masses, for the most part compact, are present within and about the cells; and some of these are to be found smeared out thin enough to see that they are composed of the minute Paschen bodies. The acetic acid evidently fixes the cells, at least partially,

so that in smearing they do not rupture to spread their content of corpuscles. The action of trypsin has the opposite effect, in that it tends to disassociate cells and to rupture them; consequently dispersed Paschen corpuscles are extremely abundant in the smears from tissue treated with it.

Discussion

It has recently been shown that the specific cellular inclusion (Bollinger body) of fowl-pox is composed in large part of uniform granules which correspond in their morphology, numbers and staining reaction with the Borrell bodies previously demonstrated in smear preparations. Furthermore, these specific inclusions have been isolated, washed and inoculated, both whole and in fractions, into chickens, with resulting successful infections.[7] Thus it has been shown that the cellular inclusion of fowl-pox carries the infectious agent; and the evidence is very strong that this agent is morphologically represented by the Borrell granules.

In molluscum contagiosum the specific inclusions have also been shown to be composed of minute granules (Lipschütz granules) which correspond in size, numbers and staining reaction with the Borrell granules of fowl-pox. Owing to the sticky consistency of these intracellular masses it has not been possible to isolate them and prove their infectiveness, although in all other respects, including resistance to tryptic digestion, the Lipschütz granules resemble the Borrell corpuscles.[8]

In these two viral infections, characterized clinically by a pox, it has been determined that the specific cellular inclusions are composed in large part of uniform corpuscles which are small enough to be filterable and numerous enough to account for the infectiousness of great dilutions of original material.

Variola-vaccinia is also characterized by a pox, and there are specific cellular inclusions (Guarnieri bodies) in the lesions. Furthermore, Paschen and others have demonstrated the great constancy of minute corpuscles (Paschen corpuscles) in smears from early lesions. These granules have the same size and staining qualities as those of Borrell and Lipschütz, and they have been shown to be agglutinable by vaccinal immune serum (Paschen, Ledingham[9]). Up to the present time, however, it has not been possible to determine any relation between the Guarnieri bodies and the Paschen

corpuscles, although Ewing [10] was able to demonstrate in Klatsch preparations a granular composition of some of these structures in cells from the rabbit's cornea. By analogy with fowl-pox and molluscum contagiosum one would suspect that the former might be in part composed of the latter. Our experiments seem to show that this is the case.

Observations which support this conclusion depend upon the fact that vaccinal infection of the chorio-allantoic membrane of chick embryos results in the appearance of unusually abundant and large Guarnieri bodies, and in extremely numerous Paschen bodies. In smear preparations from these lesions it has been possible to demonstrate structures which are interpreted to be Guarnieri bodies partially disintegrated within the cells, so that their component granules can be seen readily.

Although Paschen has frequently observed the corpuscles of vaccinia inside cells in smear preparations, he has not been able to relate them definitely to the Guarnieri body. He interprets them, however, to be the infectious agent and judges that they multiply in part, at least, inside the cells.

There is thus strong evidence, not only that the viruses of vaccinia, fowl-pox and molluscum contagiosum are cytotropic in the usual sense of having an especial affinity for cells, but that they are *cytotrophic*, if we may use this term to mean that they require under natural conditions an intracellular environment for their growth.

There is an interesting variation in the cellular affinity of the three viruses under consideration, brought out in the case of two of them by infection in the chorio-allantoic membrane of the chick embryo. Molluscum contagiosum has not yet been successfully engrafted upon a host other than man, but so far as one knows, it affects only and specifically ectodermal epithelial cells in this natural host.

Fowl-pox, readily inoculable upon the chick membrane, affects both ectodermal and entodermal epithelium, although the latter is changed relatively slightly. Vaccinia, on the other hand, affects cells of the three germinal layers, ectoderm, mesoderm and entoderm. In our studies it has been shown for the first time that Guarnieri bodies may occur not only in ectodermal and entodermal epithelium,[11] but also in endothelial cells and fibroblasts. There is thus an increasing latitude of cellular affinity as we pass from mol-

luscum contagiosum, restricted in its growth to ectodermal epithelium, through fowl-pox, which will grow apparently only in ectodermal and entodermal epithelium, to vaccinia, which finds its growth requirements satisfied in cells of all three germinal layers.

This fact no doubt has a bearing upon the cultivability of these viruses in tissue culture. Molluscum contagiosum would probably require a culture of ectodermal epithelium to initiate its growth. We have not succeeded in cultivating fowl-pox in cultures of mesodermal cells of the chick, and the cytology of its lesions suggests that it needs either a culture of ectodermal or of entodermal epithelium. Many investigators, however, have been able to cultivate vaccinia virus through several generations in a medium containing mesodermal cells of the chick, and our investigations confirm, from a cytological standpoint, the possibility of a generation of vaccine in either endothelium or fibroblasts.[12]

The method of infecting the chorio-allantoic membrane of chick embryos with vaccine offers an opportunity for further studies upon the Paschen corpuscles which appear in the lesions in great numbers, and it also provides a means of generating large quantities of sterile vaccinal virus.

Summary

1. Vaccinal lesions of the chorio-allantoic membrane of chick embryos are described.

2. Guarnieri bodies have been demonstrated for the first time in mesodermal cells (endothelium and fibroblasts).

3. Evidence has been presented that the Guarnieri bodies are composed in part of Paschen corpuscles.

4. Similarities between Borrell corpuscles (fowl-pox), Lipschütz corpuscles (molluscum contagiosum) and Paschen corpuscles (vaccinia) are pointed out.

5. It is suggested, on the basis of the cytology of the lesions, that the virus of molluscum contagiosum would require a culture of ectodermal epithelium to initiate its growth outside the body; fowl-pox would require either ectodermal or entodermal epithelium; while vaccinia would multiply in a culture of cells from any of the three germinal layers.

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DESCRIPTION OF PLATES

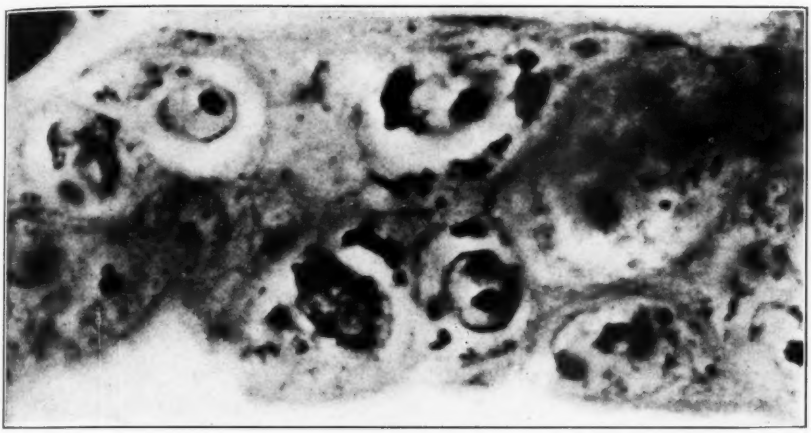
All photomicrographs taken at a magnification of 1800 diameters.

PLATE 42

FIG. 1. Epithelial cells of ectoderm showing large irregular Guarnieri bodies.

FIG. 2. Epithelial cells of entoderm showing more typical Guarnieri bodies.

FIG. 3. Epithelial cells of ectoderm showing irregular Guarnieri bodies diffusely distributed through the cytoplasm.



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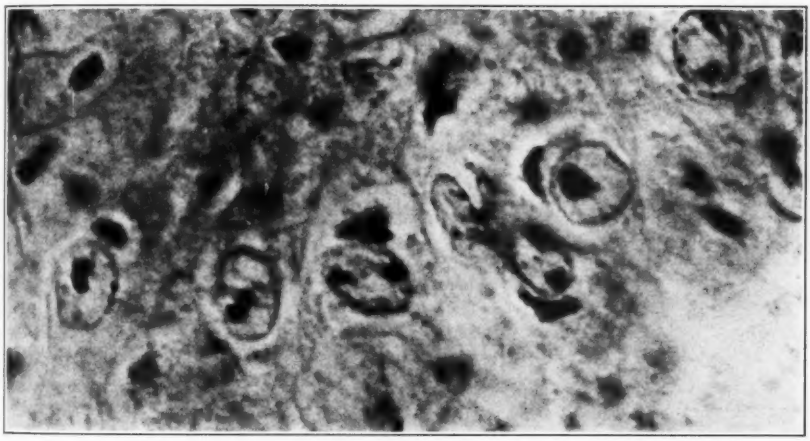

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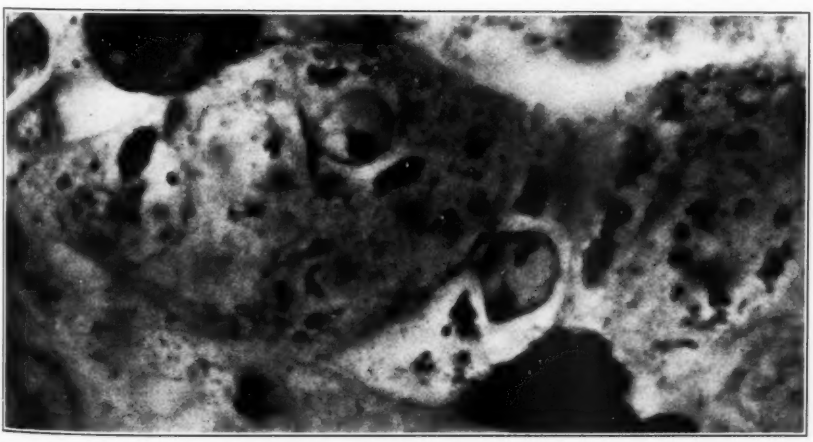


3

Goodpasture, Woodruff and Buddingh Vaccinal Infection of Chorio-Allantoic Membrane

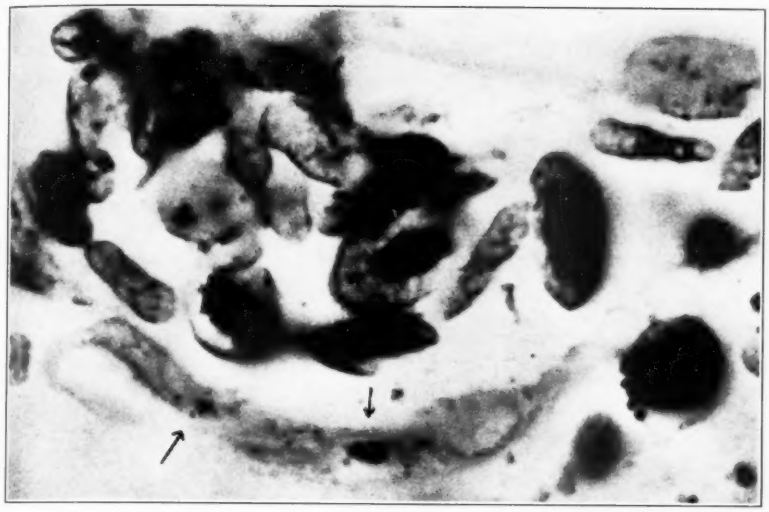
PLATE 43

FIG. 4. Vein showing two Guarnieri bodies within lining endothelial cells.

FIGS. 5 and 6. Guarnieri bodies in capillary endothelium.

FIGS. 7 and 8. Guarnieri bodies in fibroblasts.

FIG. 9. Endothelial "nodule" showing Guarnieri bodies.



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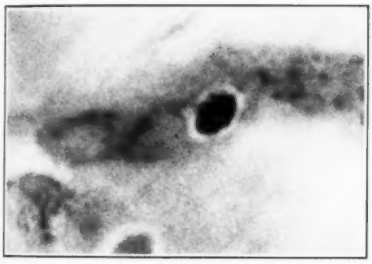

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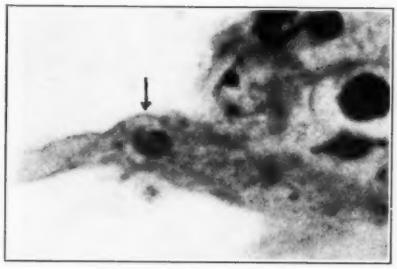

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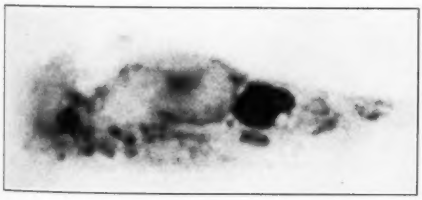

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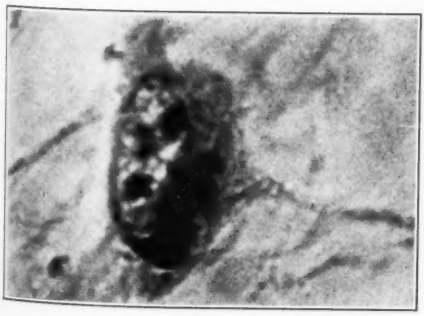

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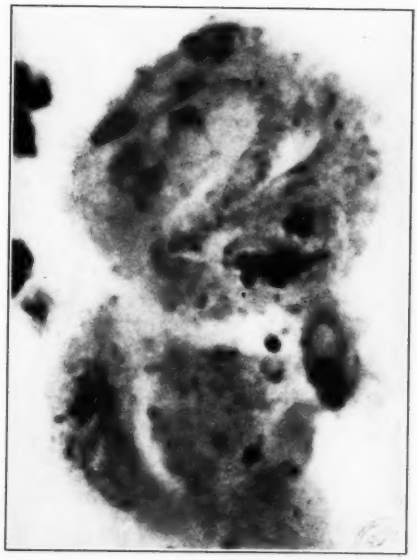


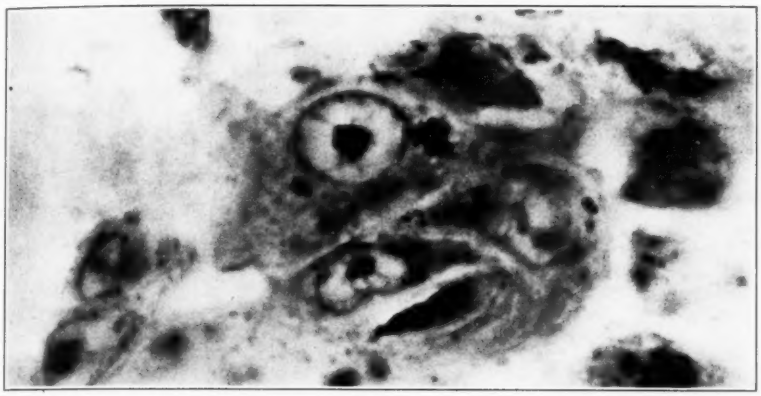
9

Goodpasture, Woodruff and Buddingh Vaccinal Infection of Chorio-Allantoic Membrane

PLATE 44

FIG. 10. Endothelial "nodule" showing Guarnieri bodies.

FIGS. 11 and 12. Cells in smear preparations showing unresolved intracytoplasmic masses, which in thinner portions are seen to be composed of Paschen corpuscles.



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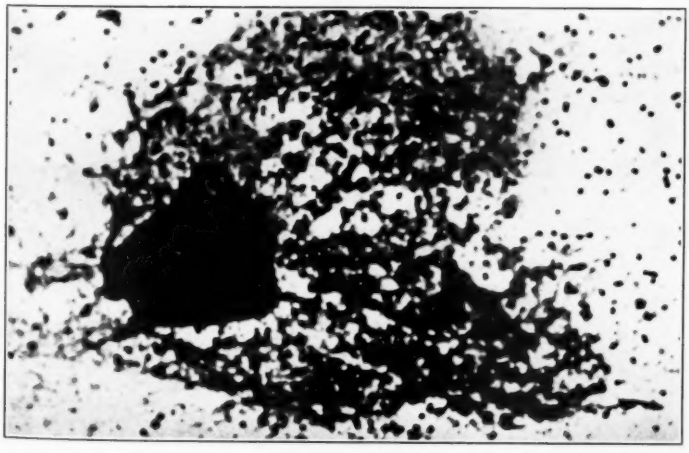

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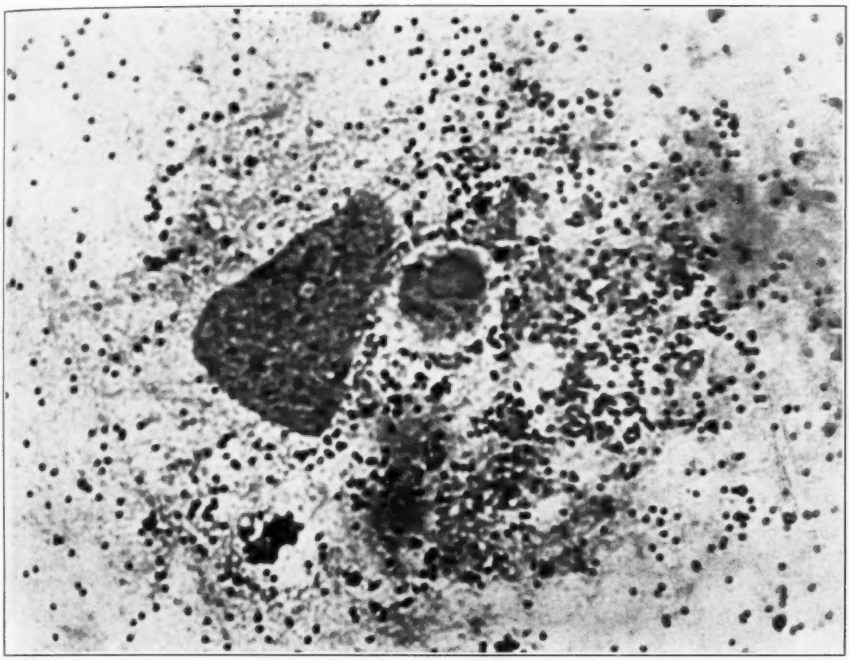
PLATE 45

FIGS. 13 and 14. Cells from a smear preparation showing unresolved and resolved intracellular groups and masses of Paschen corpuscles. K_2 filter.



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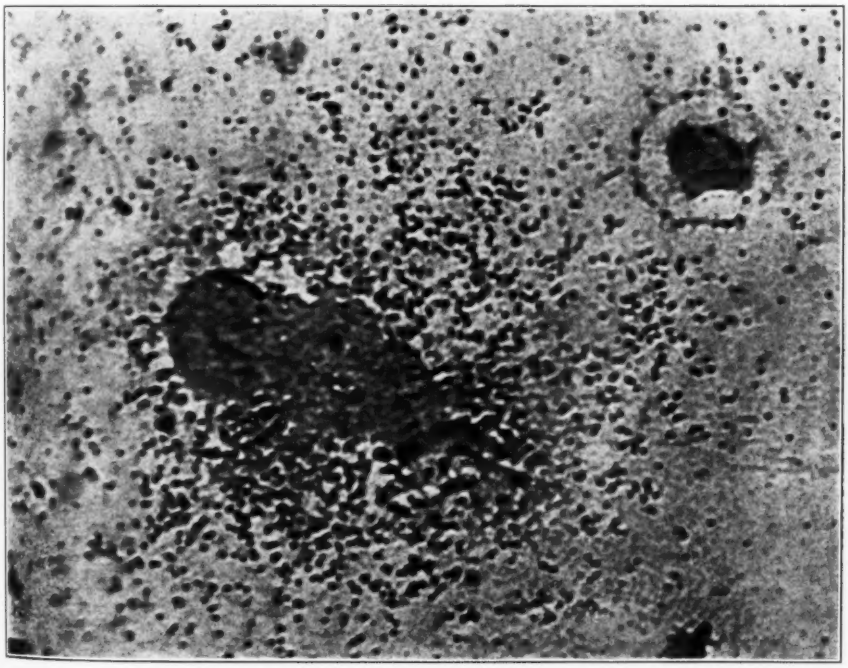

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Goodpasture, Woodruff and Buddingh Vaccinal Infection of Chorio-Allantoic Membrane

SUBCUTANEOUS NODULES IN CHRONIC ARTHRITIS *

CLINICAL, PATHOLOGICAL AND BACTERIOLOGICAL STUDIES

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Nodules varying in size and consistence are commonly found in the loose connective tissue beneath the skin in individuals with acute rheumatic fever. These nodules have been seen repeatedly by different observers and have come to be looked upon as a characteristic clinical and pathological finding in this condition.

Hillier [1] in 1868 was one of the first to describe these lesions. Meynet [2] in 1875 first pointed out that they bore a direct relation to acute rheumatic fever. Coates and Coombs [3] considered subcutaneous nodules the most specific manifestation of rheumatic fever.

Not many observations have been reported concerning the frequency and structure of subcutaneous nodules seen in chronic arthritis. Hawthorne [4] described subcutaneous nodules in six patients and considered rheumatic fever and rheumatoid arthritis different manifestations of the same process. Garrod [5] also observed subcutaneous nodules in chronic arthritis. Wick [6] saw a relation between the nodules found in chronic arthritis and those seen in acute rheumatic fever. Subcutaneous nodules in cases of chronic arthritis were also described by Coates and Coombs, Freund,[7] and Dawson and Boots.[8]

If it should be shown that subcutaneous nodules are found as frequently in chronic arthritis as in acute rheumatic fever, and that the structure and etiology of the nodules in the two diseases are similar, important data would be supplied concerning the etiology of chronic arthritis. Help might also be obtained toward classifying the chronic arthritides from the etiological standpoint.

The object of this paper was to study the frequency, structure and bacteriology of subcutaneous nodules seen in chronic arthritis,

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and to compare these nodules with those occurring in acute rheumatic fever.

Clinical Data

Material and Method: The patients studied were, for the most part, those who during the past year came to the outpatient department of the Medical School at the University of Minnesota for treatment of chronic arthritis. Subcutaneous nodules were carefully sought in 200 consecutive patients with chronic arthritis. The nodules were studied in respect to frequency, location, size, shape, consistence, development and duration. Nodules from twenty patients were removed and examined grossly. They were then cut into two or more parts under sterile conditions. A portion was fixed for microscopic study and another portion was cultured for bacterial growth. The histological structure of subcutaneous nodules found in chronic arthritis was compared with the structure of nodules of acute rheumatic origin and with experimental streptococcic nodules from rabbits.

Frequency: Dawson and Boots stated that the incidence of subcutaneous nodules in the 200 cases of "rheumatoid" arthritis examined by them was about 20 per cent. They believed that this might be higher than the general average, inasmuch as patients with subcutaneous nodules were sent to them because of their known interest in these lesions. Cecil [9] referred to them as being present in from 3 to 4 per cent of the cases.

In our series of 200 patients with chronic arthritis, subcutaneous nodules were found in fifty-nine (29.5 per cent). We have not attempted to determine the relative frequency of the nodules in the different classes of non-specific chronic arthritis, since we have not been able to group our arthritic patients satisfactorily into different distinct classes such as rheumatoid arthritis (atrophic, proliferative), and osteo-arthritis (hypertrophic, degenerative). Forty-eight of the fifty-nine individuals with nodules were 50 years of age or older. Some of these cases presenting definite nodules would be classified by many as osteo-arthritis (hypertrophic, degenerative). The following history illustrates such a case:

Mrs. F. P., aged 75 years, complained for the past five years of joint pains involving the toes, ankles, knee, right hip, fingers, wrists and cervical spines. The onset of illness was gradual, with moderate pain and stiffness in multiple joints. A definite nodule 1 cm. in diameter was found overlying the ulna 2 inches

below the olecranon process. This nodule showed microscopically a structure similar to that found in other cases of chronic arthritis and acute rheumatic fever. The X-ray report of the right knee, right wrist, fingers, ankles, toes and cervical spines was "hypertrophic arthritis" in all except the ankles, which showed no change.

The individuals with nodules did not fall into any definite group and could not be distinguished in any way from a large number of arthritics without nodules.

Symptoms: The patients were frequently unaware of the presence of the nodules. In a few instances there was slight pain at times, especially in the nodules over the elbows when they were traumatized. Three persons having nodules on the plantar surface of the foot complained of pain in the location of the nodule and walked with a decided limp, which was definitely relieved after the nodules were removed. In one person a large nodule on the dorsum of the hand caused difficulty in extending the fingers.

Duration: In most cases it was difficult to determine the duration of disease since the patients had not known of the presence of the nodules. In a number of persons the nodules were known to have been present from a few months to as long as fifteen years. A few individuals described what appeared to have been nodules, which spontaneously disappeared after having been present from a few months to a few years.

Distribution: The nodules in this series were found chiefly on the extremities. They were not searched for as carefully on the trunk and spine in all individuals. There would seem to be no reason why nodules might not occur in such locations. Two patients had nodules in bilateral arrangement over the sacro-iliac joints. Nodules frequently occurred bilaterally in the same location, especially over and below the elbows. In one individual there were nodules similar in size over the dorsum of each hand. These nodules were attached to the lower surfaces of the tendons running to the little fingers. In another person nodules of the same size were found on the dorsolateral surfaces of both feet, just behind the little toes. Twenty-four of the fifty-nine patients had a more or less symmetrical bilateral distribution of the nodules. This was especially true over the elbows.

Nodules were frequently multiple in the same location, especially over the olecranon processes, about the finger joints and over the tibias. They were found much more frequently on the upper ex-

tremities than on the lower. In fifty-nine patients nodules were found in seventy-seven locations (excluding bilateral symmetrical occurrences). Sixty-three of these locations were on the upper extremities, twelve on the lower extremities and two over the sacro-iliac joints. The distribution was as follows:

Location	Incidence
Upper Extremities	
Olecranon processes	28
Fingers (over joints)	13
Below elbows	12
Dorsum of hand	7
Palmar surface of hand	2
Fingers (dorsum and between joints)	1
Total	63
Lower Extremities	
Tibia (upper half)	4
Dorsum of foot	4
Plantar surface of foot	2
Knee (outer and lateral aspects)	2
Total	12
Trunk	
Sacro-iliac joints	2

Gross Appearance: On clinical examination the nodules were found to vary in diameter from 5 mm. or less to 3 cm. (Figs. 1 and 2). Those occurring over the olecranon processes tended to be small and those located below the elbow were frequently large. On superficial examination all were movable and not attached to the skin, but some were fairly firmly attached to the underlying tissues. On palpation most of the larger nodules were cystic and in some of the cysts a number of firm, disconnected masses of tissue could be felt. These masses within the cysts were occasionally attached to tendons. This was especially true on the dorsum of the hand.

The nodules were generally easily removed. They often had a definite capsule but in some instances this was poorly defined. When sectioned and examined grossly, multiple areas of necrosis surrounded by fibrous tissue were usually seen. Necrotic and mucinous material could frequently be expressed from the center. In none of the cases did we find the nodules calcified, as reported by Wick.

The nodules were grossly not unlike those sometimes occurring with syphilis and referred to as juxta-articular nodules. Subcutaneous nodules in syphilitic patients coming to the outpatient department have been found to be rare.

Microscopic Structure of Nodules

A comparative study was made of the structure of subcutaneous nodules seen in acute rheumatic fever, of nodules produced experimentally by injecting streptococci in small doses subcutaneously into rabbits, and of nodules found in individuals with chronic arthritis.

Subcutaneous Nodules in Acute Rheumatic Fever: Hirschsprung [10] in 1881 gave the first microscopic description of subcutaneous nodules found in patients having acute rheumatic fever. The nodules which he studied consisted of different modifications of connective tissue cells which varied in size and shape. The cells were irregular and spindle-shaped, and contained one or more vesicular nuclei larger than those found in ordinary granulation tissue. There was a homogeneous ground substance between the cells. The number of blood vessels was increased. He considered the nodule a localized area of inflammation which had a tendency to undergo necrosis.

Barlow and Warner,[11] Swift,[12] and Clawson, Bell and Hartzell [13] observed that the cellular reaction in a rheumatic subcutaneous nodule was similar to that found in the heart valves in acute rheumatic endocarditis. Cavafy [14] called attention to the presence of proliferative endarteritis in the nodules from acute rheumatic fever. This endarteritis sometimes became extensive enough to close the lumen of the vessels entirely.

The subcutaneous nodules were found by Futcher [15] to consist of fibrous tissue in various stages of development, and the cellular element to be made up of small round cells, fibroblasts, polymorphonuclear leucocytes and giant cells. Some of the giant cells contained as many as twenty-six nuclei.

Frank [16] observed peripheral and central zones in the nodules. The central area was a homogeneous mass which stained red with eosin. He decided that this homogeneous material was fibrin. The peripheral zone consisted partly of spindle cells and partly of epithelioid cells. There were numerous leucocytes. He noted variation

in structure in different nodules and considered this variation to be due to different stages of development. He considered the primary reaction in the nodule an exudate of polymorphonuclear leucocytes, which was followed by a wandering in of round cells and by a proliferation of the surrounding connective tissue.

Swift described the nodules as being made of a conglomerate number of smaller nodules. The cellular structure was similar to the structure of nodules found in the heart and other parts of the body. Clawson [17] studied nodules of acute rheumatic fever histologically in serial sections. The nodules under low magnification give the impression of multiple confluent granulomas. They are composed of multiple inflammatory foci of similar structure. The inflammatory reaction is chiefly proliferative and polyblastic in type, *i. e.*, fibroblasts and polyblasts are the most conspicuous cellular elements. Many of the polyblasts are multinucleated. Some polymorphonuclear leucocytes are found and there are irregular areas of necrosis.

Experimental Streptococcic Subcutaneous Nodules: Areas of polyblastic inflammation were observed by Small [18] in a papule developing at the site of an intradermal injection of a streptococcic vaccine.

In previous experiments, in a relatively high percentage of rabbits which had been injected intradermally and subcutaneously with strains of streptococci, Clawson [19] produced small, nodular, polyblastic lesions that were similar in the character of the cellular reaction to the subcutaneous nodules found in rheumatic inflammation in man.

Workers who have studied the subcutaneous nodules agree that the nodules consist of proliferating connective tissue cells and a cellular exudate of lymphocytes, plasma cells and polymorphonuclear leucocytes in varying numbers. In the center of most of the nodules there is some necrosis.

Subcutaneous Nodules in Chronic Arthritis: There have not been many microscopic examinations of subcutaneous nodules of chronic arthritic origin. Wick studied the histological structure of subcutaneous nodules from chronic arthritis and acute rheumatic fever, and found a marked similarity in the two conditions. Coates and Coombs compared the histological structure of nodules from acute rheumatic fever, from chronic rheumatoid arthritis, from Still's disease, and from a case of subacute bacterial endocarditis. They decided that the structure of the nodules in these conditions was

closely related, if not identical. Freund also gave a description of the microscopic structure of nodules in cases of chronic arthritis and discussed the relation between rheumatic fever and rheumatoid arthritis. Dawson and Boots observed in their fourteen cases of rheumatoid arthritis areas of central necrosis, which they thought were due to a gelatinous swelling and disintegration of collagenous bundles. They found this necrosis to be more extensive than that found in the nodules of acute rheumatic fever. They also found fibrin and an inflammatory cell infiltration in the areas of central necrosis in some instances. Large mononuclear cells were situated around the necrotic centers, generally in a radial arrangement. They considered these cells and their arrangement to be responsible for the characteristic appearance of the lesions. The blood vessels showed subendothelial deposits of fibrin, hyperplasia of the subendothelial cells with narrowing of the lumen, and perivascular cell infiltration by large and small round cells.

We have examined microscopically subcutaneous nodules removed from twenty cases of chronic arthritis. The structure in general is similar to that described by Dawson and Boots and others.

The nodules removed are found in most instances to be made up of multiple inflammatory areas (Fig. 3). The centers of these areas commonly show varying stages of necrosis (Fig. 4). In the smaller nodules only necrotic nuclear changes such as pyknosis and karyorrhexis are most commonly seen. In the larger nodular, necrotic areas the cellular structure has disappeared. Two types of structure are seen in the necrotic centers, a hyaline, eosin-staining material and a fibrillar substance that stains with hematoxylin. Scattered in this necrotic material are varying numbers of polymorphonuclear leucocytes. Surrounding the necrotic centers there are many mononuclear and multinucleated cells (polyblasts). These vary in size and shape. Many of them resemble the epithelioid cells in a tuberculous lesion. These polyblasts generally, but not always, have a marked tendency to be arranged in a radial or palisade fashion (Figs. 5 and 6). In this respect the arrangement is similar to that so commonly found in the heart valve in acute rheumatic endocarditis. A hyalinized material, similar to that seen in the valve in acute rheumatic endocarditis, is often seen (Fig. 3). Scattered among the larger and irregularly shaped polyblasts are small polyblasts. Polymorphonuclear leucocytes are scattered among the polyblasts, in many cases in small

pockets or abscesses. The nodules in these cases of chronic arthritis simulate abscesses more closely than the acute rheumatic nodules which we have studied. These necrotic, abscessed areas, since they contain streptococci, would probably tend to bring about a state of hypersensitiveness to streptococci in the patient. The small blood vessels in practically all cases show a marked perivascular increase in polyblasts, polymorphonuclear leucocytes and small lymphocytes. In some vessels the walls are thickened and the endothelium has swollen to the extent of almost obstructing the lumen. Depending apparently upon the age of the nodules, there are in most instances varying degrees of fibrosis around and about the smaller, nodular, granulomatous or necrotic areas.

The structure of the nodules from chronic arthritis does not differ in any respect, except degree, from that described in the nodules in the subcutaneous tissues, joints, tendons, galea aponeurotica, diaphragm, tongue, tonsils, arteries, heart valves, and auricles and ventricles of the heart in acute rheumatic fever. It is the structure so commonly found in acute rheumatic fever, or in nodules produced experimentally in animals by injecting streptococci. The cellular reaction in the three conditions seems probably to be due to the same cause. This type of cellular reaction is found in other streptococcic infections. The necrotic and cellular reactions and the vascular changes can be produced experimentally in rabbits and monkeys by injecting streptococci of low virulence in small numbers. The fact that subcutaneous nodules structurally similar to subcutaneous nodules in acute rheumatic fever are found in so high a percentage of cases of chronic arthritis strongly suggests a common etiology, at least in most cases.

Bacteriology of Subcutaneous Nodules

Diplococci were demonstrated in smears from acute rheumatic subcutaneous nodules by Poynton and Paine.[20] Costa [21] was able to culture streptococci from rheumatic nodules. Irish [22] obtained material from rheumatic subcutaneous nodules by puncturing them with a needle. Of the six cases examined by this method he obtained streptococci in three. Swift,[23] in describing reactions produced in rabbits by injecting streptococci, referred to strains isolated from subcutaneous nodules from acute rheumatic fever. Leichtentritt [24] found streptococci in one of three nodules cultured.

Previous to this report we attempted to culture bacteria from three subcutaneous nodules of rheumatic origin, but failed to obtain growth. Billings, Coleman, and Hibbs,[25] found *Streptococcus viridans* in a "fibroid nodule" from a patient with chronic arthritis. The location of the nodule was not given in their report. Aside from this report, no positive cultures from subcutaneous nodules in chronic arthritis have been reported. Wick was not able to obtain organisms from the nodule which he cultured. Dawson and Boots cultured nodules from fourteen patients with chronic arthritis, but had negative findings in all cultures.

We removed the nodules under sterile conditions from twenty of our patients. Seventeen of these were cultured. The nodules were brought to the laboratory immediately in sterile test tubes. They were then placed in sterile Petri dishes and cut into parts with sterile scissors. The part to be cultured was further macerated by cutting it several times with the scissors. The macerated material was then placed in a test tube containing about 10 cc. of beef infusion broth, which was similar to that used for culturing the blood from patients with acute rheumatic fever and chronic arthritis. This broth had a reaction of pH 7.6 and contained 0.2 per cent of dextrose. The material in the broth was incubated at 37° C and examined by smear preparation about three times a week. Growth, when present, occurred as a rule in less time than in blood cultures; however, cultures were not discarded until they had been incubated for at least one month. Of the seventeen nodules from different patients cultured by this method streptococci were recovered in twelve (70.6 per cent). *Staphylococcus albus* was obtained from one. Some of the strains grew well and some grew poorly. Morphologically the strains as a rule were diplococci, though two strains grew out into chains of diplococci. All were Gram-positive and produced green discoloration faintly when grown on a sheep blood agar plate for twenty-four hours at 37° C.

Summary and Discussion

Two hundred patients having chronic arthritis were studied to determine the frequency of subcutaneous nodules in this disease. Nodules varying in diameter from 5 mm. to 3 cm., and in number from one to several per patient, were found in 29.5 per cent of the cases.

Structurally these nodules seemed to be similar to those found in acute rheumatic fever and to those produced in animals by injecting streptococci. They tended to be larger in chronic arthritis. The microscopic structure was chiefly polyblastic in character, but small areas of necrosis and abscess formation were commonly seen.

When these nodules were macerated under sterile conditions and cultured in beef infusion dextrose broth, diplococci morphologically and culturally similar to those commonly found in the blood of patients having acute rheumatic fever or chronic arthritis were isolated in 70.6 per cent of the cases.

The frequency of the subcutaneous nodules in acute rheumatic fever and chronic arthritis, the similarity of the gross and microscopic structure of the nodules in these two conditions and in experimental streptococcic nodules, and the frequency with which streptococci can be cultured from the blood in acute rheumatic fever and from the blood and nodules in chronic arthritis, strongly suggest that acute rheumatic fever and chronic arthritis for the most part have a common streptococcic etiology and that the two diseases are in all probability different manifestations of the same process.

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DESCRIPTION OF PLATES

PLATE 46

FIG. 1. Subcutaneous nodule on arm below elbow.

FIG. 2. Large subcutaneous nodule on right side of foot.

1

2

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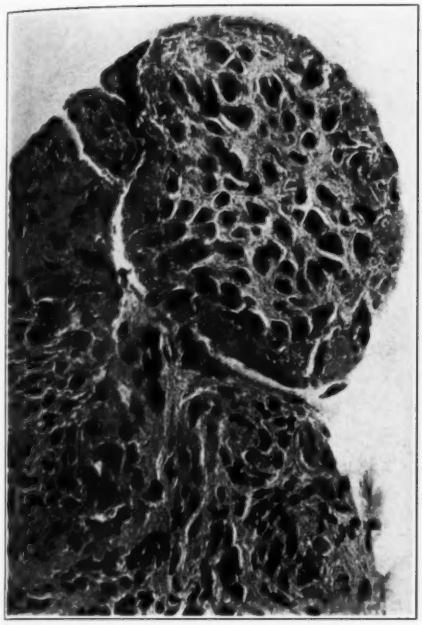
PLATE 47

FIG. 3. A small subcutaneous nodule and part of a larger one lying near each other. The smaller nodule shows a hyalinized material and cellular content similar to that in a valve in acute rheumatic endocarditis.

FIG. 4. A nodule showing a necrotic center with radial arrangement of polyblasts around the necrotic area.

FIG. 5. Cellular content of a nodule against the necrotic center on the right.

FIG. 6. Palisade arrangement of cells against the necrotic center in the nodule.



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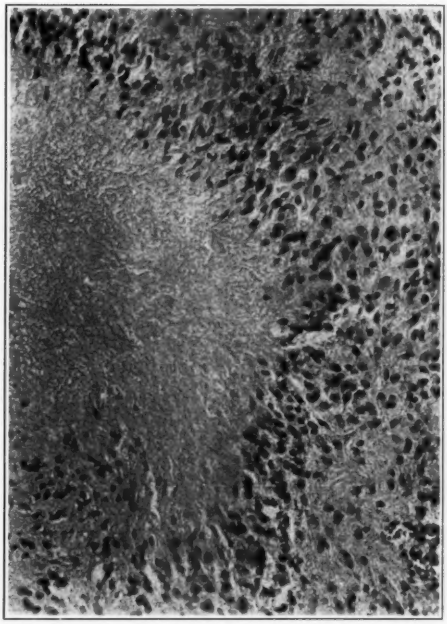

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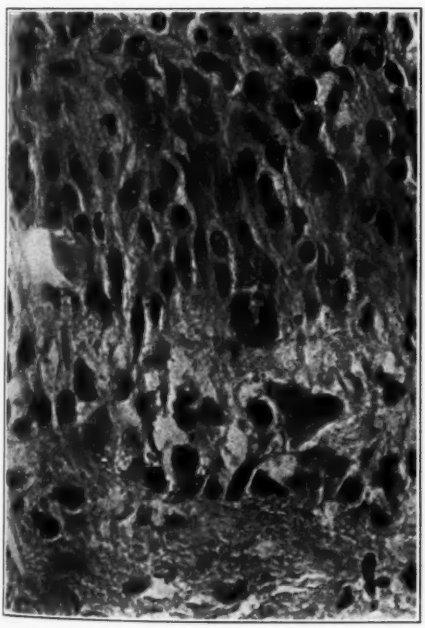

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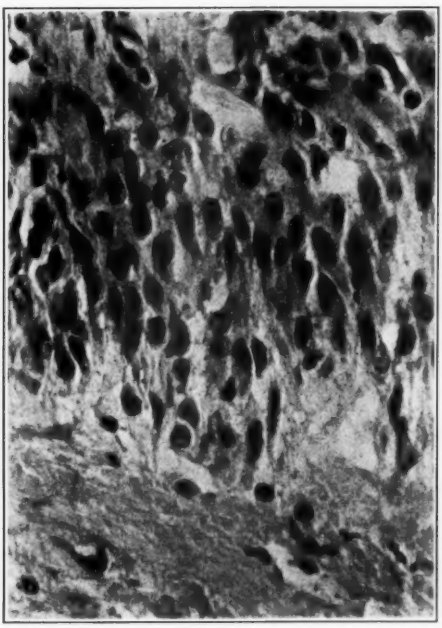

6

Clawson and Wetherby Subcutaneous Nodules in Chronic Arthritis

A METHOD FOR PROGRESSIVE SELECTIVE STAINING OF NISSL AND NUCLEAR SUBSTANCE IN NERVE CELLS *

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The usual staining methods for the demonstration of Nissl substance suffer from a variety of technical defects and difficulties. I have succeeded in devising a progressive selective method for staining this substance, which I believe to be of both practical utility and of some theoretical importance.

TECHNIQUE

The Nissl substance is a constant histological element in nerve cells, which has a marked affinity for basic dyes. As yet, however, every method for demonstrating it has depended upon the "regressive principle of staining," *i. e.*, overstaining and then differentiating in alcohol. The basic anilin dyes have been the most useful, and yet generally speaking they do not stick firmly enough to the tissue elements and are too readily removed by alcohol. In this respect there is only a difference of degree between these various dyes. It is clear that the differentiation profoundly influences the results, and the hurry with which the preparation must be passed through the alcohols and xylols is a great disadvantage. Following an extensive study of the literature I arrived logically at the following method, which eliminates nearly all of the above difficulties.

Becher [1] introduced the use of certain dyes from the groups of anthraquinones, naphthoquinones and oxazines. These dyes are combined with a metallic element forming a soluble "lack," and this now basic dye becomes bound by the fixed acid nuclei (see also Romeis [2]).

The dyes † we have used are:

1. Naphthazarin (1, 2 — dioxynaphthoquinone).
2. Alizarincyanin R (1, 2, 4, 5, 8 — pentaoxyanthraquinone).

* Received for publication January 8, 1932.

† Nos. 1, 3 and 4 were obtained from Dr. Hollborn (Grübler-Hollborn, Leipzig), and No. 2 from Dr. G. Grübler & Co., Leipzig.

3. Gallocyanin (oxyproduct of the oxazine group).
4. Gallamin blue (derivative of No. 3).

As lack-forming salts we used:

$Al_2(SO_4)_3$
K_2SO_4, Cr_2SO_4, 24 H_2O (chromalum).
$Al\,Cl_3$
K_2SO_4, $Al_2(SO_4)_3$, 24 H_2O (potassium alum).
$Na_2\,B_4\,O_7$ (borax).

The salt is dissolved in distilled water, whereupon the dye is added and mixed thoroughly with the solution. The mixture is then warmed gradually and gently and finally allowed to boil for 15 to 25 minutes, the bottle being shaken frequently. After gradual cooling and filtration the solution is ready for use. In the case of Naphthazarin and Alizarincyanin R the solution is allowed to stand for about 8 days and then refiltered.

The following dye solutions were employed:

1. Naphthazarin-aluminum sulphate 5%, pH = 3.23
   $Al_2(SO_4)_3$ ..... 10. gm.
   Distilled water ..... 200. cc.
   Naphthazarin ..... 0.35 gm.
   Color of solution: intense, deep reddish violet.
   Staining time: 20 to 40 hours.

2. Alizarincyanin R-aluminum sulphate 5%, pH = 3.43
   $Al_2(SO_4)_3$ ..... 10. gm.
   Distilled water ..... 200. cc.
   Alizarincyanin R..... 0.3 gm.
   Color of solution: deep violet with reddish tint.
   Staining time: 20 to 30 hours.

3. Alizarincyanin R-aluminum chloride 5%, pH = 3.09
   $Al\,Cl_3$ ..... 10. gm.
   Distilled water ..... 200. cc.
   Alizarincyanin R ..... 0.3 gm.
   Color of solution: deep violet with a somewhat stronger red than Solution 2.
   Staining time: 20 to 30 hours.

4. Alizarincyanin R-chromalum 5%, pH = 1.87
   $Al\,Cl_3$ ..... 10. gm.
   Distilled water ..... 200. cc.
   Alizarincyanin R ..... 0.3 gm.
   Color of solution: dark blue with a gray tint.
   Staining time: 24 to 60 hours.

5. Gallamin blue-potassium alum 5%, pH = 2.43

Potassium alum	10.	gm.
Distilled water	200.	cc.
Gallamin blue	0.4	gm.

Color of solution: deep, dark blue.
Staining time: 24 to 48 hours.

6. Gallocyanin-chromalum 5%, pH = 1.84

Chromalum	10.	gm.
Distilled water	200.	cc.
Gallocyanin	0.3	gm.

Color of solution: deep, intense blue, with a faint violet tint.
Staining time: 24 to 48 hours.

7. Gallocyanin-chromalum 2%, pH = 1.89 (*i. e.* chromalum 4 gm.)
Color of solution: approximately the same as in Solution 6.
Staining time: 24 to 48 hours.

8. Gallocyanin-borax 2.5%, pH about 9 (*i. e.* borax 5 gm.)
Color of solution: deep, intense blue; after standing for about 12 days, a marked reddish brown tint appears.
Staining time: 24 to 48 hours.

The pH of the solutions was determined with a quinhydrone electrode. The absolute values are less significant than the constancy for each solution and the differences between them. The figure for Solution 8 is naturally inexact, the solution being too alkaline for this method.

The animals used were killed by cutting both carotids under light ether anesthesia and allowing the animal to bleed to death. The desired part of the central nervous system was quickly exposed and small pieces (about 5 to 8 or 10 mm. long) cut out and immediately put into the fixing fluid.

For fixation were tried:

1. 96% alcohol, after the method of Nissl.
2. Alcohol-formalin (90 vol. 70% alcohol plus 10 vol. of the usual 40% solution of formaldehyde after being neutralized).
3. Sublimate-alcohol.
4. Formol-alcohol-sublimate (F. A. S.) (1 vol. 40% formalin, 4 vol. 93% alcohol, 5 vol. saturated aqueus solution of sublimate).
5. Zenker's fluid with acetic acid.
6. Neutral formalin (1 part formalin, 4 parts distilled water).
7. Susa (Heidenhain).

The fluids Nos. 1 to 4 gave excellent results and with a little longer duration of staining No. 5 also gave splendid results. No. 6 gave the same excellent pictures, but No. 7 gave a somewhat weaker staining.

In No. 1 the tissue was fixed up to maximum consistency in 5 to 6 days with frequent changing of fluid, in No. 2 about 24 to 48 hours, in No. 3 — 24 hours, in No. 4 — 8 to 12 hours, in No. 5 about 20 hours (medulla oblongata of dog), in No. 6 about 3 to 4 days. Then followed the usual procedures for dehydration, and so on. All the material was embedded in paraffin through methylbenzoatcelloidin and benzol after the method of Péférfi. The slides (sections 5 to 10 microns thick) are placed directly from distilled water into the dye solution in question. By varying the duration of staining the intensity of color can be graded somewhat, but overstaining practically never occurs. In general, old solutions (several months) require a longer time for staining than fresh ones.

After staining, the specimen is carefully washed in distilled water, dehydrated in alcohols of increasing concentrations in xylol, and mounted in balsam, damar-resin or cedar oil. When the stained slides are run through the alcohols there is no danger of their losing color. The staining is absolutely alcohol-stable and differentiation does not occur.

Description of Results

Figs. 1, 2, 3, 5 and 9 illustrate the results obtained with Solutions 1 and 2. Stained with Naphthazarin (Solution 1), the Nissl granules appear blue-violet, and more deeply colored than with Alizarincyanin (Solution 2) where they are reddish and not so distinct. The nerve cell in Fig. 9 appears faint and somewhat washed out. In Solution 1 the co-staining of the interstitial protoplasm of the single nerve cells, as well as of fibrillar structures, is violet, in Solution 2 more reddish. Otherwise the intensity of the co-staining seems to be approximately the same in both instances. Examining Fig. 5, where the staining time is so much longer, the co-staining is correspondingly greater than in Figs. 1 and 2. Also, the interstitial protoplasm and the karyoplasm in general (Kernsaft) are more strongly colored, yet the nucleolus is easily distinguishable. The picture appears more diffuse. On the whole the Naphthazarin method yields a very good staining for general orientation and for the arrangement of the Nissl substance.

With Alizarincyanin (Solution 3) we get a much more intense color (Fig. 10). The Nissl substance is a deep red-violet color, the karyoplasm is relatively light, the nuclear membrane can be seen.

The interstitial protoplasm of the nerve cell is strongly stained in the same reddish color but, owing to the intense darkness of the tigroid, the method yields a certain contrast which makes it fit for use. The glia nuclei are markedly colored, but do not stand out particularly because of the strong co-staining of fibrillar structures. The general impression of this staining is the impurity of the picture as a whole.

With Solution 4, where the same dye is in combination with chromalum, one gets a picture of entirely different character, both in color and intensity. After 53 hours of staining (Fig. 11), glia nuclei, the Nissl substance and nucleoli of the nerve cells are but faintly light blue or water-blue. There is also a weak co-staining of the interstitial protoplasm of the nerve cells and of fibrillar structures. Practically the staining is not of much value.

Gallamin blue (Solution 5) yields a fine, pure, bright blue staining of the tigroid (Fig. 12). The staining comes next to the Gallocyanin-chromalum in color and purity, but the blue co-staining is considerably more extensive and of approximately the same intensity as with Naphthazarin. Yet the staining is of practical value.

By using Gallocyanin (Solution 6) one gets Nissl pictures of a quality unapproached by any other method. I have tried and used all of them, Nissl's original method included. Even the finest, most carefully made thionin or toluidin blue specimens do not approach the Gallocyanin specimens, the force of which lies in the progressivity and selectivity of the staining. The Nissl substance (Figs. 4, 6, 7 and 8) appears deeply stained in the purest and finest blue color, giving an excellent sharp contrast against the practically unstained interstitial protoplasm. The nucleolus stands out sharply from the pale nucleus. Glia nuclei are also beautifully stained, giving a good contrast against the pale background. In other words, the general co-staining of fibrillar structures, and so on, is practically minimal. A glance at the figures shows the superiority of this method to those previously described. The beautiful appearance of the nuclear cap (Kern Kappe) in Fig. 4 is noteworthy. On examining the pathological case of Fig. 8 one notices how sharply and beautifully every detail is brought out — the central degeneration with tigrolysis, accumulation of chromophile material at the periphery of the cell, the vacuolization, and so on.

Any of the usual methods of after-staining may be applied to specimens stained by the methods we have described. For example, we may use Alizarin-red S for 3 to 8 minutes, followed by washing in distilled water and so on; or erythrosin (0.05 per cent) or eosin (0.05 per cent) for 5, 15 or 60 seconds, followed by washing and differentiation in graded alcohols and so on. We hope that this staining method, being so easy and simple, may prove of practical value, particularly to neuropathology.

Somewhat similar staining experiments have been made by Kihn [3] but with totally negative results. His work has even been quoted as showing the great limitations of this staining principle as applied to the nervous system. Just what factors underlie his negative results, I am so far unable to tell. Apparently he has used solutions containing only 2 per cent chromalum. Therefore I tried Solution 7. Macroscopically the slides show a stronger staining as compared to specimens from the same block stained in Solution 6; apparently more dye has been absorbed. Microscopically this is due to a greater general co-staining, especially of fibrillar material. In the individual nerve cell there is also a somewhat stronger staining of interstitial protoplasm and the karyoplasm, but there is no alteration in the staining of the Nissl substance itself. This possibly indicates (the pH being the same in both cases) that the dye has become less completely bound to the chromalum to form the compound dye-lack, and consequently is to a certain extent acting as an acid dye.

The alkaline solution (8) was tried on two differently fixed preparations from the spinal cord of a rabbit. The first specimen fixed in formalin-alcohol looks macroscopically as if there were no staining at all. Microscopically the only things that are brought out are the Nissl bodies and nucleolus of the nerve cells and the glia nuclei. These appear as weak, gray shadows, faint, but distinctly visible. Sometimes one also sees the nuclear membrane. The other preparation, fixed in trichloracetic acid, gives an entirely different picture. Macroscopically the slides appear relatively deeply stained — a uniform gray-blue. Microscopically there is considerable general staining of fibrillar material, and so on. The protoplasm of the nerve cells is gray, and the nucleus is considerably darker with a clearly distinguishable nucleolus. There is not a sign of Nissl bodies. This illustrates very well the significance of the fixative and the well known importance of its acidity or alkalinity.

An alteration in the pH of the dye solution was also tried. In order to do this N/10 HCl or NaOH were added to the Gallocyanin-chromalum solution, the determination of pH made quickly, and immediately afterward the specimen in question put into the solution for staining.

Solution 6–(original) ..... gives pH = 1.84
Solution 6–55 cc. plus N/10 HCl 30 cc. ..... gives pH = 1.58
Solution 6–40 cc. plus N/10 NaOH 15 cc. ..... gives pH = 3.57
Solution 6–42 cc. plus N/10 NaOH 35 cc. ..... gives pH = 4.57

In doing this the dye solution was diluted, but this does not seem to be of any great importance in this connection. The results, though revealing nothing that could explain Kihn's negative results, were rather interesting. With fixative No. 2 we find macroscopically a characteristic gradation of color intensity, the most acid solution giving by far the lightest, the least acid solution giving the darkest stain. The amount of stain absorbed in general by the tissue is, as one would expect (see Pischinger [4] 1926), a function of the pH of the dye solution, all preparations having been fixed in the same way. Microscopically one sees that this is due to the general co-staining. In the specimen stained in the solution of pH = 1.58 there is practically no co-staining at all. With the usual solution of pH = 1.84, the co-staining is just perceptible. pH = 3.57 gives a considerably stronger co-staining, and pH = 4.57 still greater. Between the first and the last solutions there is an enormous difference, but the most interesting thing is the comparatively slight change in the staining of the Nissl substance itself, in striking contrast with the general co-staining. That is to say, the specific tigroid and chromatin staining is practically unaffected by this alteration in pH of the dye solution. The results with fixative No. 1 are almost identical.

In general, the compound Gallocyanin-chromalum, being a basic dye, is more readily absorbed in a less acid solution than in a more acid one, this effect manifesting itself microscopically through alterations in the general co-staining of the tissue which is clearly a direct function of the pH of the solution; whereas the specific Nissl and chromatin picture is relatively unaffected within the same range of variation of pH.

Discussion

To indicate the theoretical significance of this method in regard to the origin and composition of the Nissl substance we need consider only a few necessary data from the literature on the subject. It will appear that the color reaction of the Gallocyanin is not entirely devoid of theoretical importance.

Mackenzie [5] found that the Nissl substance contained iron, and finally stated definitely that it was an iron-holding nuclear chromatin. Held [6] found that it gave a positive reaction for phosphoric acid, and classed it as a nucleoproteid. Scott,[7] applying the methods of Macallum, found the Nissl substance to give a positive reaction for both iron and phosphorus. On the basis of his embryological investigations he further states that the iron-containing material (Nissl substance) is gradually formed by transfer of nuclear substances through the nuclear membrane into the cytoplasm during the course of development. The nuclei of embryonic nerve cells he found to be much richer in basophil chromatin material than after the Nissl substance had become differentiated. Recently Nicholson,[8] using Macallum's hematoxylin test for iron, has been able to show that the distribution and appearance of iron-containing material in the cytoplasm is practically identical with the distribution and appearance of the Nissl substance. Previously von Lenhossek [9] had said that the tigroid is already present in the young neuroblasts in the form of smaller and more diffusely arranged spheroidal masses. Collin [10-12] confirms Scott's observations and states that he has been able to observe in his preparations all the phases involved in the process of transfer of chromatin material from the nucleus into the protoplasm. He further finds that the chromatophil substance first appears in the immediate neighborhood of the nucleus of the nerve cells of chick embryos of about six days' incubation. Marcora [13] states that the differentiation of chromatophil granules is first observable on the tenth day of incubation, always appearing first in the peripheral part of the cellular protoplasm. Beyond this he does not confirm Collin's observations, and he believes that the question of the nuclear origin of the chromatophil substance cannot yet be solved. According to van Biervielt's investigations on human material (cited from Marcora) the first Nissl granules become differentiated in the third month, appearing at the cell periphery; but the differentiation is by

no means perfect until in the seventh month of fetal life. Cowdry[14-17] definitely states that the Nissl substance belongs to the category of chromidial substances in general, and infers that it is formed as the result of nuclear activity. All the investigators seem to agree that the Nissl substance appears relatively very late in development.

Mott [18] has been able to confirm the ultramicroscopic observations of Marinesco [19-22]. After vital staining and treatment with formalin he showed further that the cytoplasm became filled with minute blue granules. He therefore draws the conclusion that the minute refractive granules make up the basophil substance which forms the Nissl substance (Schollen), while Marinesco himself considers them to be identical with the neurosomes of Held. The Nissl figures (Schollen) as such, both Mott and Marinesco consider to be artefacts. On the contrary Stöhr,[23] on the basis of investigation with ultraviolet photomicrography, claims to have observed in fresh condition the same localization and grouping of the Nissl granules in the cytoplasm as in fixed and stained preparations. This is also confirmed by Weimann.[24]

De Moulin,[25] using as fresh material and as well adapted technical arrangements as possible, added methylene blue to his emulsion and found no basophily of the cytoplasm, but the nucleus as well as nucleolus became deeply stained. Gradually the nucleus lost its stain, while the cytoplasm, on the other hand, gained in color and slowly became a diffuse homogeneous blue. Granulations then appeared, and at last regular Nissl figures (Schollen). His conclusions are that postmortem changes in the degree of dispersion in cell and nucleus occur, causing a shift in the fluids; and following lesions of the nuclear membrane, the basophil colloids of the nucleus get into the protoplasm where they coagulate, and thus finally form the Nissl substance. The latter he therefore considers as an artefact composed of nuclear substances.

Recently a systematic criticism has been made of the methods for microchemical detection of phosphorus, and therefore Scott's results must be considered with some reservation (see Bethe [26] and Policard and Leulier [27]). Bielschowsky,[28] and several others, deny any connection between nuclear substances and the Nissl granules. The most outspoken critics are Unna and Gans.[29] They state that the substances widely distributed in epithelial and connective tissue cells, and described by Unna as "Granoplasma" are also present in

nerve cells and have been long known there as Nissl bodies. As a support of this view they refer to some cases of identical behavior of these substances in epithelial cells, plasma cells, liver and nerve cells. As a supplement to these positive points they give the negative microchemical proof that the Nissl bodies do not contain nuclein. The basis of this proof is their assumption that purified methyl green is an absolutely reliable reagent for distinguishing between nuclein and other acid protein substances in the cell; but the lack of specificity of this reaction has been pointed out (see Cowdry [15]). They also find that Nissl substance dissolves in warm water (65°) in twelve hours and believe it to be albumose (cytose).

Heidenhain [30] has advanced a very interesting and stimulating hypothesis on the biological function of the Nissl substance, based on the assumption that it is derived from the nucleus and contains chromatin substance, and he suggests the name "Cytochromatin" for the Nissl granules.

Stimulated by Heidenhain's hypothesis and assuming that Mackenzie, Held, Scott and Nicholson were essentially correct in their views as to the chemistry of the Nissl granules, it seemed to me quite plausible that the Nissl substance should manifest an affinity for, and be stainable progressively with, some of the purest and finest nuclear dye-lacks from the group of anthraquinones, naphthoquinones and oxazines. Our results, which demonstrate just such an affinity, serve to support the assumption that the Nissl substance is related to chromatin, and to oppose the views of Unna and Gans. In this connection I wish to emphasize the distinct gradation in the character, intensity and purity of the staining according to what dye combination is being used. This gradation shows a distinct parallelism between the quality of the staining of true nuclear chromatin material and that of the Nissl substance, *i. e.*, *the purer the affinity for nuclear chromatin the stronger appears to be the affinity for the Nissl substance.*

The problem of the nature of Nissl substance presents the following alternatives. It may be (*a*) a product of nuclear activity formed during the life of the developing neurone and fulfilling a function of importance in the metabolism of the neurone; or (*b*) a preformed material (präformierten Stoff, (Unna)) arising outside of the nucleus during ontogenesis, able to reform or regenerate during life; or (*c*) an artefact containing nuclear substances (de Moulin). This

problem I leave open for the present, being engaged in further investigation upon this question.

Conclusions

Well aware of the danger and difficulties of deducing from mere similarity of dye affinities the identity or chemicobiological relations of two tissue elements, I nevertheless believe that the positive Gallocyanin reaction furnishes new evidence that the Nissl substance really contains nuclear chromatin substances. The results of the chemical, the embryological and the experimental investigations summarized above, and certain characteristics of the Gallocyanin reaction, definitely lend support to such a view.

The specific Nissl staining of the Gallocyanin-chromalum is practically unaffected within a very wide range of pH of the dye solution, and also in combination with borax in an alkaline solution it still stains the chromatin and Nissl substances feebly but definitely. This indicates that possibly some chemical affinities are involved in this special staining process. Further investigation of this point is in progress.

The cytological work leading to the method described here was started during my stay in the Anatomical Institute of Munich, continued in Doctor Vimtrup's laboratory in Copenhagen, and greatly extended and completed in the Marine Biological Laboratory of Woods Hole this summer.

I wish to express my gratitude to the director of the Anatomical Institute of Munich, Geheimrat Professor Mollier and to Prosector Doctor Vimtrup for their great hospitality and supply of material. Also I wish to thank Doctor Neel, the director of the Psychiatric Laboratory of Copenhagen, for his kindness in supplying me with vast, special neuropathological material upon which I have tested the practical pathological validity of the staining method.

Above all I want to express my deep gratitude to my friend and teacher Dr. Robert Feustel of Munich for his many valuable suggestions, stimulating ideas and never-failing interest.

And last I wish to express my thanks to Dr. Hallowell Davis for his revision of this paper and valuable advice.

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DESCRIPTION OF PLATES

PLATE 48

FIG. 1. Cells from the spinal cord of a rabbit; formalin-alcohol, Naphthazarin-$Al_2(SO_4)_3$. Staining time: 23 hours, 25 minutes. × 860.

FIG. 2. Anterior horn cell of a rabbit; formalin-alcohol, Naphthazarin. Staining time: 23 hours, 25 minutes. × 860.

FIG. 3. Purkinje cell, from the cerebellum of a rabbit; neutral formalin, Naphthazarin. Staining time: 23 hours, 25 minutes. × 860.

FIG. 4. Purkinje cells, cerebellum of a rabbit; neutral formalin, Gallocyanin-chromalum. Staining time: 22 hours. × 860.

CONCERNING THE HISTOLOGY OF MELANOMA *

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INTRODUCTION

Certain features of melanotic tumors, noted in frozen sections impregnated with silver, were discussed at some length in a recent paper (Foot [1]). The point most stressed was the presence of a very complex reticulum in the tumors examined, the fibers of which were described and reproduced photomicrographically. The origin of these fibers, their probable nature and their ultimate distribution, were points reserved for later and fuller discussion, as it was not then possible to impregnate paraffin sections satisfactorily, and these are indispensable. Now that a very satisfactory method has been devised, it is proposed to carry the discussion farther and to supplement that which has already been said with a description of new data that have resulted from the study of thinner, more perfect sections. Before entering into the details of the matter, it is advisable briefly to review the subject in respect to that which has already been advanced by another investigator, whose work inspired these studies.

Two articles published by Masson [2] in 1926 have covered this theme so thoroughly that, in checking over his work by means of other methods than his, one may at best hope for little more than a confirmation of his findings. The more one reads his papers, the more one is impressed with their completeness and clear-cut logic. His conception of the histology of the pigmented nevi and of their malignant congenors, the melanoblastomas, is none too readily grasped at first reading and it will do no harm to review it here.

Masson believes with Soldan [3] that the pigmented nevi are nervous, not dermal tumors; their type cell, according to him, is that of the sheath of Schwann. It can differentiate in various directions: in the superficial portions of the tumor beneath the epidermis it takes on a cellular, more or less epithelioid form, lying in nests that resemble Meissner corpuscles on the one hand and the smaller groups

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of Merkel-Ranvier cells on the other (in the former case the cells are large and pale, in the latter they are smaller, polyhedral and possessed of protoplasmic processes); in the deeper layers of the tumor these cells become elongated into fusiform, or even filamentous structures that are closely applied to the nerve fibers and may be flattened into concentric plates that are, in turn, enclosed in a collagenous envelope or endoneurium that ramifies among them and gives them an onion-like, laminated appearance on cross-section. When the tumor cells become so attenuated as to be filamentous he calls them "nevus fibers," which is rather misleading and apt to be misunderstood, as they are really merely elongated cells rather than true fibers. Transitions between the superficial and deep types of growth may be observed in the intermediate layers of the tumors.

Such newgrowths, then, lie directly in the course of the terminal branches of the cutaneous nerves and are, therefore, closely related to neurofibromas. The pigmented cells, formerly considered so important as to give their name to the tumors, he explains on the basis of a changed metabolic activity; the fact that two or more types of cell contain melanin is no proof that they are necessarily genetically related. In support of this he cites the presence of melanin-bearing cells in other parts of the nervous system. The part played by the specialized Merkel-Ranvier cells of the epidermis is explained by the assumption that they are outposts of the tactile apparatus — potential outposts of nevi — intercalated between the true epithelial cells of the basal layer and of another cell race than these. He speaks of all the tumor cells as constituting an overgrowth of the "peripheral neuroglia," doubtlessly basing his terminology upon the theory that the Schwann cells are of epiblastic, neuroglial origin.

Having thus outlined his views as to the histogenesis of the tumor, he indicates very clearly that it is unprofitable to attempt any further classification of its subtypes, as was formerly the fashion. If his conception be understood, it will readily be seen that tumors developing in the deep layers of the skin will take on the complicated neurofibromatoid type of growth, while those arising just beneath the epidermis will tend to be cellular and alveolar, the type cell varying as it inclines now toward those of the Merkel-Ranvier group, now toward those of the Meissner corpuscles. Tumors developing in both the pars papillaris and the derma will show a mix-

ture of the two types, with a transition zone in between. This is no uncommon picture. He demonstrates nerve trunks, sometimes quasinormal, sometimes distorted by the proliferation of their Schwann cells, entering the tumors at their bases and becoming intricately involved in the cell complexes more superficially located. They then emerge from these to continue to a point beneath the epidermis, where they branch out into the subepidermal plexus. He also notes the fact that erector pili muscles are often directly involved in the tumor growth, sometimes appearing to share in it; this he explains on the basis that they develop from the same primordium. He carries these theories a step further and applies them to malignant nevi, "melanosarcomas" and "melanocarcinomas," as well.

In my previous article referring to results obtained with frozen sections impregnated with silver, no special mention was made of the tumor cells other than to point out the utter dissimilarity between them and the true epithelial elements of the epidermis. The paper concerned itself chiefly with the fibrils that were so strikingly demonstrated in silver impregnations, structures that Masson interpreted as collagenous fibers. That they are not ordinary connective tissue fibers, but rather endoneurium or actually nerve filaments, was indicated. It was shown that they were only imperfectly impregnated if the usual potassium permanganate-oxalic acid bleach was used before impregnating, whereas the reticulum and collagen of the stroma were, on the other hand, well silvered. Preliminary bromuration of the sections (calculated unfailingly to demonstrate astrocytes and neuroglia fibrils) produced much the same effects — the fibrils of the nevus nests were not impregnated, although the reticulum of the stroma was brought out sharply. It was seen that the finer fibrils of the nevi differed from those of a fibrosarcoma which were indubitably reticulum or collagen, and no such structures were demonstrable in sections from an epidermoid carcinoma, indicating that they were not of epidermal origin in the nevus or they would be present in the carcinoma also. Other stains failed to demonstrate them specifically; they were not fibroglia, neuroglia or elastic tissue.

There was, therefore, nothing definite to show just what these fibrils were, although several methods showed quite plainly what

they were not. It remained to continue with the work, using more perfect and diverse methods of silver impregnation and any other procedure that might afford a clue as to their true nature. Accordingly, a method of impregnating the finest branches and end twigs of the fibers in thin, paraffin sections was worked out after considerable experimentation. This is detailed in another paper to which the reader is referred (Foot and Foot [4]).

Technique

Two variants of the silver method just mentioned (which will hereinafter be called the "p. g. s." method for sake of brevity) were used chiefly in the examination of a number of pigmented nevi of types which varied in respect to situation, melanin content, malignancy or non-malignancy, and so on. These were Variants 2 and 5; other variants sometimes proved themselves to be helpful. Various stains were employed to supplement these, and the Ranson-Cajal and Bielschowsky reduced silver impregnations, both slightly modified, were resorted to in order to impregnate the neurofibrils more specifically. It was found that the methods of mass impregnation which depend upon the use of silver diammino hydroxid tend to deposit too much silver in the epidermal layers and to underimpregnate the deeper ones, unless great care is exercised. The very portion of the block that one most particularly desires to examine seems to be the one in which there is the least detail and densest precipitate.

Ranson-Ramon y Cajal Method: Tissue blocks are fixed in absolute alcohol with 1 per cent ammonium hydroxid for 48 hours; they are then rinsed for ½ to 3 minutes in distilled water, depending upon the size of the blocks. After being transferred to pure pyridin for 24 hours they are washed in many changes of distilled water for another 24 hours and then set in the dark in a 2 per cent solution of silver nitrate in distilled water at 37° C for 3 days. After rinsing in distilled water they are placed in a 4 per cent solution of pyrogallol in 5 per cent neutral formalin, made up with distilled water, for 1 to 2 days, after which they are rinsed in water and embedded in the usual manner in paraffin. The sections are a pale canary yellow, the neurofibrils are black, the endoneurium yellow and the myelinated fibrils old-gold, surrounded by a color-

less sheath. This method was very slightly modified by adding the Laidlaw oxalic acid-gold toning procedure. The deparaffinized sections were toned in 1:500 gold chlorid for 5 minutes and redeveloped in 5 per cent oxalic acid for a like period, washing between steps with tap-water; they were then fixed in 5 per cent sodium thiosulphate and washed well in water, after which they were mounted in the usual way in Canada balsam.

Bielschowsky Method: The blocks are fixed in 10 per cent neutral formalin, washed and treated for 3 to 4 days with pure pyridin. They are then washed in running water until there is no more odor of pyridin, and further washed in a few changes of distilled water to remove any chlorides. They are transferred from this to a 3 per cent solution of silver nitrate in distilled water for 3 to 5 days in the dark at 37° C, after which they are rinsed in distilled water and placed in a solution of silver diammino hydroxid in the dark at 37° C for 24 hours. This solution is made up by adding strong ammonia dropwise to 10 cc. of a solution of silver nitrate in distilled water of 10.2 per cent strength, until the resulting precipitate is just dissolved; 10 cc. of 3.2 per cent pure sodium hydroxid in distilled water is then added and the resulting reprecipitation again just dissolved with ammonia. The solution is then made up to 100 cc. with distilled water. The blocks are next washed in several changes of distilled water for 2 hours and then placed in 20 per cent neutral formalin in distilled water for several hours. After washing in water, they are then embedded in paraffin in the usual way. They are toned in gold chlorid intensified in oxalic acid, fixed in sodium thiosulphate (as above described) and mounted in Canada balsam. Further experiments with the Bielschowsky method, calculated to decrease its intensity without lessening its penetration, are now under way, but they are not wholly satisfactory in their present form.

The Ranson-Cajal method gives the most uniform results, but it is also apt to be somewhat uneven in its results.

Cellular Morphology

Nuclei: These are rather vesicular and may contain one or more nucleoli which are more prominent in the malignant than in the non-malignant members of the group.

Cytoplasm: When studied by ordinary methods the appearance of the cytoplasm is quite misleading; the epithelioid character of the cells, as noted in hematoxylin-eosin sections, is too familiar to need discussion. With silver methods their true morphology is brought out, and Masson's schematic plan is confirmed in every particular; the type cell is polygonal in its primitive form (Fig. 1), with short, hair-like processes. The coarser, tail-like processes are seen better in reduced silver sections where the cytoplasm impregnates more deeply than the nuclei (Fig. 2). They may also be demonstrated in the "p. g. s." method if Bouin's fixative is used instead of formalin, in which case the impregnation is reversed from a "positive" to a "negative" picture, as in the case of the Laidlaw method [5] after Bouin fixation.

The more differentiated cells become, in this way, racquet-shaped; they develop one or two stout cytoplasmic "tails" and, by continuing the process, pass through a fusiform phase into enormously elongated, filamentous structures. Specimens impregnated with Variant 6 of the "p. g. s." method often bring out a web-like, filamentous, intracellular reticulum that may be concerned in the production of the nerve sheath webs under normal conditions. The fibrils of these will be discussed further later on. In the deeper layers of the tumors the scale-like, laminated form of type cell is found in Masson's "lames foliacées" or "leaf-like lamina" (Figs. 3 and 4). In malignant melanomas one sees bizarre distortions of these type cells (Fig. 5) bearing the same relation to them as the gigantic cells of glioblastoma multiforme bear to the glioblasts (spongioblasts), or those of fibrosarcoma to fibroblasts — they are enlarged caricatures of the type cells of the benign tumors. In fact, the nevus cell, when impregnated with silver, bears a striking resemblance to a neuroglia cell although, as we have seen in a previous paper (Foot and Zeek [6]), it does not impregnate specifically in bromuration methods which unfailingly demonstrate astrocytes in frozen sections.

The Laidlaw impregnation fails to demonstrate the nevus cells satisfactorily in sections from benign nevi fixed in formalin, Bouin's or Zenker's fluids; they are at best faintly brought out and are, therefore, "Laidlaw-negative," in comparison with the epithelium of the epidermis and its adnexae, which are positive and well shown.

A strange fact is noted in this connection: the cells of malignant melanomas examined in the course of this work are usually "Laidlaw-positive." Just what this signifies is not yet evident.

The arrangement of the type cells follows Masson's description so closely that it would be futile to describe it again; his observations may be checked up in every particular. As to their melanin content, here, too, his observations may be confirmed. Some of the cells contain considerable quantities of melanin, while the bulk of their fellows are free from it. The presence of the pigment makes the racquet-like morphology of the melanoblasts more evident in those stains where the cytoplasm is not completely demonstrated, so that one might imagine that they differed from their fellows, were one to judge them by hematoxylin-eosin standards alone; when reduced silver is used, however, it becomes evident that the only observable difference between the two types is the presence of melanin in the one and its absence from the other.

Fibrillar Morphology

With such stains as the Mallory phosphotungstic acid hematoxylin, the Van Gieson stain, and so on, the fibrils appear to be ordinary collagen. Using Variant 5 of the "p. g. s." method this is also true, for it does not differentiate between collagen and reticulum; if Variant 2 be used, however, many of the fibrils come out black like the reticulum. We have seen in the two preceding papers that reliable reticulum impregnations depending upon Mallory's bleach fail to demonstrate fibrils much beyond the limits of the stroma; those that are brought out in the nests by the "p. g. s." method fail to materialize satisfactorily if the bleach has been used. Therefore, one would appear to be justified in believing that these fibrils differ in some way from connective tissue collagen or reticulum. The reduced silver methods tend to obliterate the intercellular fibrillary details and to make the fibers appear like a homogeneous matrix (*cf.* Fig. 2), but they demonstrate nerve fibrils in the trunks and the cell nests. Such neurofibrils may be theoretically traced from the nerves at the base of the tumors, through the laminated sheaths to the subepidermal, epithelioid nests. When medullated they run as pale canals with a central axone through the laminated sheath expansions; when non-medullated they appear as curving,

roughly parallel, chestnut-brown fibers that are intimately associated with the distorted sheaths formed by the proliferating tumor cells (Fig. 7). Actual connections between nerve trunks and subepidermal nests have admittedly not been traced in the form of continuous fibers which emerge from nerve trunks at the base of the tumor, continue through its intermediate cell complexes and terminate in or near the subepidermal nests; that this could be done in serial sections, given the requisite material and patience, is definitely indicated. The actual terminal distribution of the fibers is very difficult to determine; they seem to skirt the tumor cells, impinging upon them very closely, and to enter a complicated and incompletely impregnated rete in the pars papillaris of the skin. The besetting difficulty in all this work is that of determining the identity of nerve fibers, as distinguished from reticulum or collagen. All these usually stain or impregnate almost identically alike, so that it is only in the reduced silver sections that one may be at all sure of one's ground. Sometimes one may find sprouts of proliferating Schwann cells following nerve twigs into the subepidermal stroma, and in these cases, in reduced silver preparations, one may observe very delicate but intensely impregnated fibrils accompanying the cell columns and, apparently, not extending very much in advance of them.

That Masson's conception of an arborial distribution of the tumor along nerve trunks and branches is correct is everywhere indicated. If one should make tangential sections of small nevi, one may obtain some of them at levels that show mostly connective tissue of the corium; in this tissue the nerves will have been cut transversely and one will find that they are surrounded by nevus cells (Fig. 6), while these are to be seen nowhere else in the section. This is good proof that the tumor growth follows the nerve sheaths, but it does not tell us whether it is following them from below upward, or *vice versa*. Such pictures as that shown in Fig. 8, however, make it seem more probable that the former assumption is the more probable.

Summary and Classification of Fibrils

Collagen and Reticulum Fibrils: These are found chiefly in the stroma of the tumors, but there are many of them that bear a much closer resemblance to the endoneurial varieties of connective tissue

than they do to ordinary connective tissue. Their caliber is very variable, not uniform; they are often recurved upon themselves, forming sharp angles and triangular varicosities at the point of reflection, and their staining properties are in every way similar to those of endoneurium. They do not form coarse collagen bundles, as does the connective tissue, and they do not resemble the reticulum of the areolar tissue or the lymphoid tissue. In the case of the laminated sheath swellings the fibrils are of the utmost delicacy.

Neurofibrils: These have already been described at length.

Protoplasmic Fibrils: These are found *within* cell bodies; in the non-malignant tumors they are in the cell processes, where they constitute a very delicate intracellular reticulum or "web"; in the malignant forms they may run all through the cytoplasm of the cells. The attenuated cellular processes, which Masson calls "nevus fibers," scarcely merit this term and should be recognized as filamentous extensions of the cytoplasm; it should be recognized, however, that they may contain the intracellular fibrillar webs just referred to, which are well shown in the case of Schwann cells in the sheath of a normal lingual nerve. A comparison of Figs. 2, 9 and 10 will make this clear. The above exposition probably explains the reason why more fibrils are demonstrable with the "p. g. s." technique than in the case of reticulum impregnations depending upon a preliminary bleach; the group of finest fibrils in the nevus nests are probably, for the most part, of a nervous or protoplasmic rather than of a reticular or collagenous nature.

Discussion

It is, then, possible to confirm Masson's ideas regarding the histology of pigmented nevi and melanoblastomas in every particular; that the control has been made exclusively by means of silver methods that differ essentially from his trichrome stain, makes the confirmation the more striking. His conception of the morphology and distribution of the type cell, of its differentiation from a polygonal unit, through a fusiform phase to a filamentous, or (also) a flattened one, is completely borne out. His thesis as to the type cell's derivation from that of the sheath of Schwann is reinforced by the striking resemblance of the two in properly impregnated silver sections, and by the possibility of demonstrating columns of tumor cells along the

course of nerves not as yet involved in a neighboring nevus, as well as by such pictures as the one shown in Fig. 3 where the nerve is entering an obvious tumor complex. The proliferation of Schwann cells along neighboring nerves could not be illustrated in this article by reason of space demands, but excellent photomicrographs in my possession attest to the veracity of the above statement.

An examination of malignant representatives of this tumor group reveals characteristics that one might be led to expect in malignant transformations: the cells become distorted, enlarged, lawlessly arranged, poorly differentiated, and their Laidlaw reaction becomes positive instead of negative; their fibrils no longer show the excellent endoneurial type of differentiation seen in the non-malignant tumors, but tend to be finer, less similar to true nerve sheath fibrils and often predominatingly intracellular.

The importance of the melanoblast as a type cell, in the case both of malignant and non-malignant "melanomas," dwindles as one examines these; some tumors may be almost exclusively composed of melanoblasts, but many will show very few. Masson's idea that they constitute nevus cells with a predominatingly chromogenic metabolism is everywhere strongly indicated, and the unfailing presence of cells, or groups of cells among them that show no demonstrable melanin and conform to the amelanotic type nevus cell, indicate that these are the more primitive, and therefore the true type cells of the tumor, while the melanoblasts are derived from them, rather than *vice versa*.

Summary

Masson's theories that nevus cells are (1) derived from those of the sheath of Schwann, (2) non-pigmented in their pristine state, (3) associated with nerve trunks and fibers, and (4) closely related to those of the neurofibroma, are uniformly confirmed by an examination of a series of pigmented nevi by means of silver impregnations of several types.

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DESCRIPTION OF PLATES

The photomicrographs were made by Prof. Joseph B. Homan, with the assistance of the author.

PLATE 51

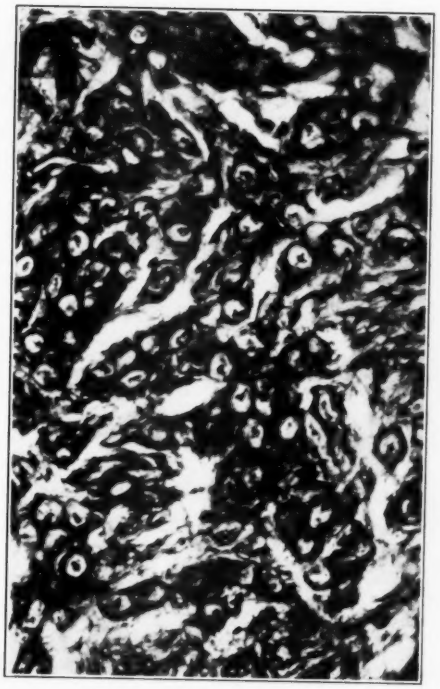
FIG. 1. Field from a subepidermal nevus nest, showing the large Meissner type cells at the top and the smaller Merkel-Ranvier type at the bottom. "P. g. s." technique, Variant 2. × 800.

FIG. 2. A similar field, to demonstrate the morphology of the cells as brought out by the Ranson-Cajal reduced silver method plus gold toning. Note the pale nuclei and dark cytoplasm. × 800.

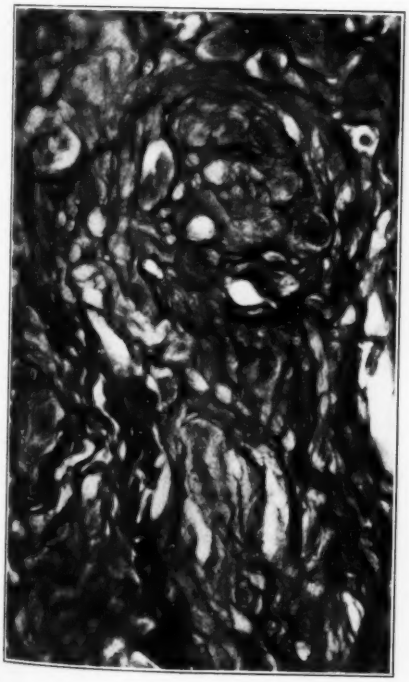
FIG. 3. One of Masson's "lames foliacées," or laminated sheath expansions impregnated to show its fibrillary endoneurium by the "p. g. s." technique. Variant 2. × 800.

FIG. 4. A similar field impregnated by the Bielschowsky reduced silver method in the block, with subsequent sectioning and gold toning. This shows the neurofibrils as well as the endoneurial fibers. The former may be seen entering the expansion at its lower pole and coursing among its cells. × 950.

1



2



3

Foot

4

Histology of Melanoma

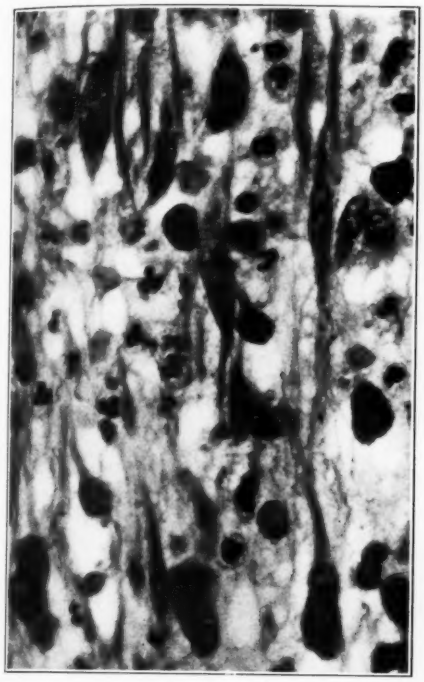
PLATE 52

FIG. 5. Distorted forms of nevus cells in a malignant melanoma shown in an edematous, inflamed portion of the tumor where their morphology is more evident on account of their spacing. "P. g. s." technique, Variant 2. × 800.

FIG. 6. A nerve trunk in transverse section penetrating a nevus nest in the derma. The axis cylinders are clearly visible. Below it is one of the distorted pseudo-Meissner corpuscles so often observed in pigmented nevi. A large axone (black rectangle) runs obliquely through it, surrounded by its clear sheath. Bielschowsky reduced silver method with gold toning. × 950.

FIG. 7. Longitudinal section of a nerve trunk running through a nevus nest. Many of its fibers appear to be non-medullated. Ranson-Cajal reduced silver method with gold toning. × 800.

FIG. 8. Topographic picture of a nerve of the corium entering a nevus at its base; just below center the nevus cells are sharply focussed, rather out of focus at the bottom. The nerve runs along the right border of the picture directly into the nests. The black masses at the upper left are collagen bundles in the corium. The section was cut tangentially with the epidermis and a millimeter or so beneath it, which demonstrates the intimate association of the peripheral nerves with the tumor which dips down along them from the upper, into the deeper skin layers. Bielschowsky reduced silver method, gold toning. × 350.



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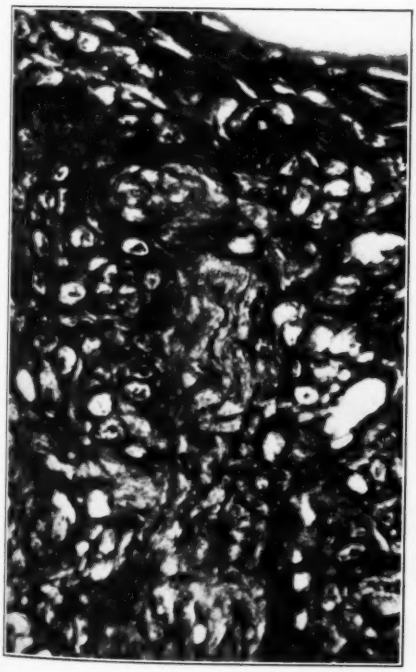

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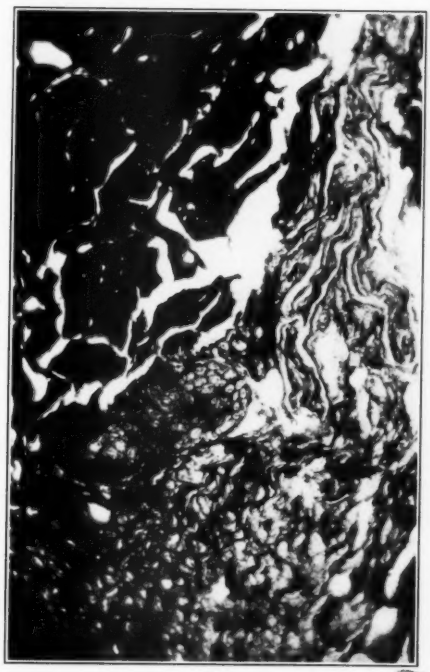

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Histology of Melanoma

PLATE 53

FIG. 9. A field to show the intermediate type of growth lying between the nevus nests and the "lames foliacées." Note the two types of cell and the intricate endoneurial fibrils. "P. g. s." technique, Variant 2. × 800.

FIG. 10. For comparison with Figs. 2 and 9; a field from a nerve in normal human tongue impregnated by a Variant 6 of the "p. g. s." method. The striking similarity of the sheath cells to the nevus cells is at once apparent. The endoneurial fibers are not as sharply brought out by this variant as in that of Variant 2 or 5, but the cells and their connection with the sheath webs is so well shown as to make up for this. × 950.

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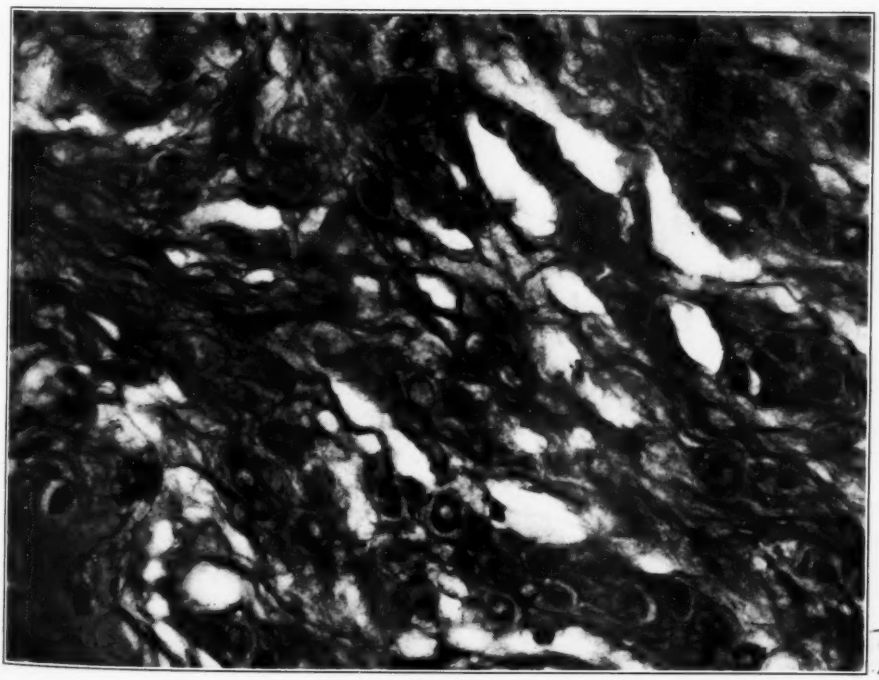

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Foot

Histology of Melanoma

CONCERNING THE HISTOLOGY OF MELANOMA *

II. WITH SPECIAL CONSIDERATION AS TO THE NERVOUS ELEMENTS OF THE TUMOR

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In the first paper under this title (Foot [1]) much evidence was advanced in support of Masson's [2] theory as to the nervous origin of melanoma, but there were a number of important points that remained obscure and required further working out, chief among them the fate of the nerve trunks that were found traversing the tumor nests, a matter that was left largely to conjecture, the supposition being that they continued out to the epidermis and broke up to join in the formation of a more or less hypothetical nervous rete in the pars papillaris. One was left with the impression that the lack of a method that would definitely and metachromatically distinguish between nerves and connective tissue fibers and would demonstrate the ultimate distribution of the former, was a distinct handicap.

Rogers' [3] technique for impregnating nerves in paraffin sections has supplied this deficiency in part, but its use has not entirely cleared up the matter, for the extremely delicate network of fibrils that surrounds the more primitive cells in the tumor nests still eludes definite classification. By applying this method, however, it has been possible to carry the investigation several steps further along its original line, reinforcing what has already been said and warranting the publication of a report on the progress thus accomplished.

As material for this report a number of benign and malignant melanomas were utilized. The benign tumors were of two distinct types: (*a*) those that were flat, brown and hairy, and (*b*) those that were more or less pedunculated and often almost non-pigmented. The malignant tumors were in part primary, in part metastatic. As normal control material, sections from a clavus of the toe and from the tip of normal human tongue were used. A small neurofibroma of the palmar surface of a finger served as additional control material and afforded interesting comparison with the melanomas.

* Received for publication March 14, 1932.

Technique

Rogers' method was followed very closely in the main, with a few slight changes in the procedure calculated to fit the case in point. In carrying out this technique it is well to wear rubber gloves, for the silver solutions are very strong and stain the fingers and hands intensely. A strong solution of potassium cyanide in water will remove these stains fairly well if used immediately, but its very poisonous nature makes it a poor and dangerous substance to have about the laboratory, and it should be employed with extreme caution.

Fixation: 10 per cent formalin is used, with a 2 per cent solution of ammonia in absolute alcohol as an alternative. Rogers also uses Bouin's fixative, and notes that either neutral or non-neutralized formalin may be employed.

Embedding: This is carried out in the usual manner, the blocks being dehydrated with 80 per cent, 95 per cent and absolute alcohol, but to each of these 2 per cent of ammonia is added. Then the blocks are kept for an hour or so in pure absolute alcohol, run through chloroform and chloroform-paraffin, and then into paraffin. After sectioning, the paraffin is removed with xylol, the sections run through absolute alcohol, and they are then kept for 12 to 24 hours in 95 per cent alcohol with 2 per cent ammonia. It was found that material embedded in the usual way, without the addition of ammonia, gave good results if the long, ammonia-alcohol treatment was resorted to after the paraffin had been removed. In fact, many of the illustrations in this article were made from sections so treated.

Impregnation: After a rinse in 80 per cent alcohol, the sections are placed in 40 per cent aqueous silver nitrate solution in the incubator for 20 minutes at 37° C, the staining-box being placed on the heating plate. This is a very concentrated solution, to be sure, but its use seems to be unavoidable. The sections are rinsed briefly in distilled water and treated with 20 per cent formalin for 5 minutes, after which they are changed into 1 per cent formalin for a few minutes. From this they are taken one by one, blotted briefly with filter paper to remove the excess formalin (but not to the point of dryness) and flooded with a few drops of a diammoniacal silver solution from a dropping-bottle. The solution is left on for 30 to 60 seconds, poured back into the bottle and the slides blotted off on

the same filter paper. The reagent is made up as follows: to a given quantity (say 20 cc.) of 20 per cent aqueous silver nitrate, strong ammonia is added drop by drop until the resulting precipitate is just dissolved; then one drop of ammonia for each 2 cc. of silver solution (in this case 10 drops) is added to afford the necessary excess, after which the solution is diluted with an amount of distilled water equal to the original quantity of silver nitrate solution (here, 20 cc.).

The blotted sections are next placed in 20 per cent formalin, where they turn bright yellow to old-gold. It should be noted that *the sections should not be washed between steps, merely blotted long enough to remove the excess reagent.* It should also be remarked that no sodium hydroxid is added to the impregnating solution, as in the case of some other methods of impregnation.

Toning: The sections are then washed in distilled water and toned for 15 minutes in a 1:300 aqueous gold chlorid solution to which 2 cc. of glacial acetic acid has been added for each 100 cc. This is said to restrain the impregnation of the connective tissue fibers without interfering with that of the nerves, thus affording better contrast. It turns the sections from old-gold to grayish, or slate. They are then intensified by a 5 minute immersion in a solution containing 2 per cent oxalic acid and 1 per cent formalin. This changes the gray color to purple and gives more colorful pictures. After washing at the tap, fixing for 5 minutes in 5 per cent sodium thiosulphate and washing once more, the sections are run up through ascending percentages of alcohol into xylol and thus to Canada balsam, in the usual way.

The connective tissue is lavender to purple, the nuclei black, the nerves highly refractile and black to brownish black, and the only source of confusion is the impregnation of the fibrils of the erector pili muscles and the delicate fibers in the tumor nests; the reticulum is poorly impregnated throughout. All efforts to improve upon this method by reducing the strength of the very concentrated solutions utterly failed. There is only one reason for attempting improvements and that is the somewhat variable results obtained during the flooding of the sections with diammoniacal silver solution. It is supposed to be heated to about 50° C, but it was found to be too powerful when heated and the melanoma sections were overimpregnated as a result. The investigator should

experiment with this step until he has obtained the desired results. Dilution, a little more or a little less ammonia, or variations in the length of immersion of the sections in the fluid may all be resorted to. The method will give brilliant results if one but perseveres in its use.

Results of the Investigation

As tactile corpuscles and nerves play the leading part in Masson's theory and as we are interested in proving or disproving this, sections of tongue, of a corn and of a neuroma were first examined to determine the value of this method. The results are shown in Figs. 1, 2, 3, 4 and 5.

In the tactile corpuscles of the skin, the nerves run in medullary sheaths to the base of the organ, lose their sheaths and appear to ramify within the capsule among the epithelioid cells, apparently terminating in extremely complicated networks (Dogiel's "Retikolaren") see Fig. 2, or in club-like expansions (Fig. 3). In the tongue the latter are more usual and one finds, in addition, bodies resembling Grandry's corpuscles (Fig. 3), composed of two cells with a nerve filament lying between their apposed surfaces. The clubbed terminals may, in some instances, lie in the connective tissue outside of the tactile corpuscles. In both skin and tongue, heavy, webbed, non-medullated fibers may be found running about in the pars papillaris (Fig. 4). These are important structures to bear in mind later on, when the same fibers are being described in the case of the tumors. As to the neuroma (Fig. 5), it is found to be made up of coiled, parallel nerve trunks with medullary sheaths and an excess of fibrous tissue between trunks. The nerves seldom tend to stray from the neural sheath and the picture is, therefore, quite different from that to be described in connection with the melanomas.

Having established some standards, let us see what is found in the pigmented nevi. The large, flat variety shows numerous acini of primitive, polygonal cells that were described in the first paper; the larger, more epithelioid "Meissner" cells are not well represented. In such tumors it is possible to demonstrate nerve trunks at the base of the growths and trunks lying within their thin, perineural sheaths in the center of tumor nests (Figs. 6, 7 and 8). Often the nerves seem to impinge upon a tumor alveolus and stop at its margin (Fig. 7), but it is occasionally possible to demonstrate nerve fibrils apparently

running into an alveolus (Fig. 8). All attempts to connect them definitely with the fine reticulum among the tumor cells failed.

The second type of mole examined, more or less pedunculated and definitely raised above the epidermal surface, shows more cells of the epithelioid type and much more resemblance to Meissner corpuscles. In some cases the nerves appear to run in the interacinar stroma, to terminate either in blunt coils or in branching, finger-like terminals with something of the appearance of abortive Dogiel endings (Fig. 9). In other instances one finds large, clubbed cells here and there that resemble the nerve terminals in the Meissner bodies, but are unconnected with nerve trunks. One also notes, among these, double groups of cells resembling Grandry bodies, but again, lacking nerve filaments. In this type of tumor it is possible to trace the medullated nerve trunks much farther out toward the epidermis than one can in the preceding type. Many of the trunks almost immediately become non-medullated and of the thick, webbed type shown in the pars papillaris of normal controls. These may show very fine forms with varicosities along their course, lying among tumor cells and coursing along their borders.

One rather large, sessile mole, that was already used in the work described in the preceding paper, shows very striking differences when compared with its fellows, with the exception of one mole that was very similar to it in its gross appearance. One is immediately struck with the presence of numerous elongated cells that impregnate deeply and are often reticulated, resembling the clubbed terminals of the nerves in the Meissner bodies. Among these one notes numerous stout fibers that impregnate in a peculiar manner and are somewhat similar to those seen in the pars papillaris of the skin; they contain vacuoles at intervals and are otherwise more compact than the webbed filaments already referred to (Fig. 10). It is at once evident, on examining the "lames foliacées" of this tumor (structures not prominent in the flat type of nevus) that these present most of the distinguishing features of Meissner bodies. The large, clubbed endings are often suggested and structures that appear to be abortive attempts at the formation of Dogiel's "Retikolaren" can be easily found (Fig. 11). In isolated instances, medullated fibers are seen traversing the laminated bodies in their long axis.

One is made increasingly uneasy, while studying all types of these benign tumors, by the very intimate association of the erector pili

muscles with the tumor cells (Fig. 12). In almost every tumor examined these were either closely surrounded by tumor cells or they were broken up so that small, atypical smooth muscle cells became intercalated between tumor cells. Masson has already stressed this close association. In one tumor strands of skeletal muscle are found near sebaceous glands, running fanwise into the tumor. Both types of muscle cell, however, appear always to be in the minority and may be merely "sympathetically" involved, as is often the case with basal cells of the epidermis over such tumors. If one attempts to revamp the scheme of the pigmented nevus on a muscular basis one does not get very far, but that we must bear in mind the possibility of this being a mixed tumor is clearly indicated.

It seems, then, that benign pigmented nevi fall into two extreme types: those poor in nervous elements and composed of primitive, polygonal cells in ovoid nests (grossly, flat tumors) and those rather rich in nervous elements and composed largely of what appear to be perverted Meissner corpuscles, in the form of Masson's "lames foliacées." Transitions between the two types are so frequent that it seems unwise to attempt a definite separation of these — they are described merely for the sake of accuracy.

The malignant tumors examined did not throw much light upon the subject — they get too far away from the rather complicated differentiation of their benign congenors for one to draw very definite conclusions concerning them. When they metastasize to lymph nodes they are even less obviously related to the benign type, for they are quite innocent of pseudo-Meissner bodies and nerve filaments. In their primary sites, however, they possess more or less similarity to the benign growths and there seems to be no reason for our recasting our theories as to the relationship of the two.

Masson derives the type cell of the tumor from that of the sheath of Schwann and this idea was adhered to in my first paper, but now that a good method for staining the nerve fibers has been found, one hesitates whether to ascribe the origin of the type cell to the Schwann cell or to those of the endoneurium and perineurium. It is very difficult to decide the question on the basis of the material at hand. Comparing melanomas with a neuroma, however, one may say that the latter consists chiefly of nerve trunks with proliferated epineural or fibrous sheath cells and interneural fibrous tissue; the

melanoma, on the other hand, appears to consist of cells connected more especially with the nerve terminals and their adnexa and, to a lesser extent, with the end filaments themselves. This bears out Masson's theory in every particular save one — the derivation of the type cell from the Schwann cell — and this cannot be refuted; it seems safer, for the present, not to be too categorical as to the derivation of the type cell, beyond saying that it is probably derived from one of the cells of the inner neural adnexa.

Summary

By means of Rogers' technique of silver impregnation, it has been possible to demonstrate nerve fibers in melanomas and to show a striking resemblance between Masson's "lames foliacées" and the normal Meissner corpuscles, not only in respect to their morphology (which Masson has already brilliantly shown), but also in connection with the distribution of nerve filaments in and about them. This article merely reinforces what was said in its immediate predecessor and supplies some of the deficiencies that were to be noted in that paper, which were due to the lack of a suitable method for attacking the problem.

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DESCRIPTION OF PLATES

All the photomicrographs were taken from sections impregnated by the Rogers' technique. They are all at 800 diameters magnification, except Fig. 2, which is about 2000 diameters. They were made by Prof. J. B. Homan, of our Department of Medical Art, with the assistance of the author.

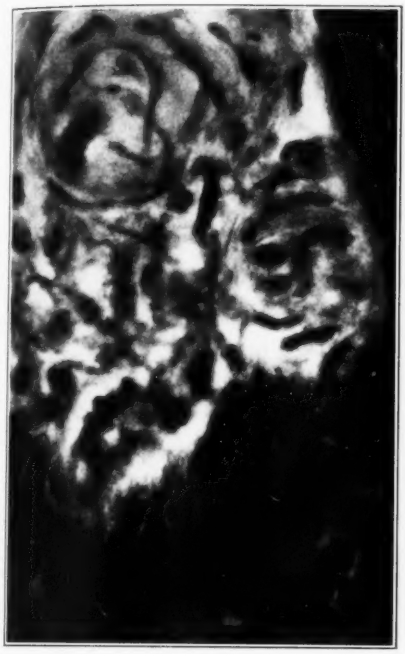
PLATE 54

FIG. 1. Meissner corpuscles just beneath the epidermis of a clavus.

FIG. 2. Oil immersion photomicrograph of a portion of a Meissner corpuscle, to show the complicated, webbed "Retikolaren" of Dogiel.

FIG. 3. Two Meissner bodies from normal human tongue, showing a somewhat simpler form of nerve terminal than that seen in the preceding pictures from the skin. Note the numerous nerve filaments in the stroma and the two reniform nuclei with a small fibril between them (Grandry body?).

FIG. 4. Reticulated non-medullated fibers from the pars papillaris of the epidermis, one fibril running across an epidermal papilla.



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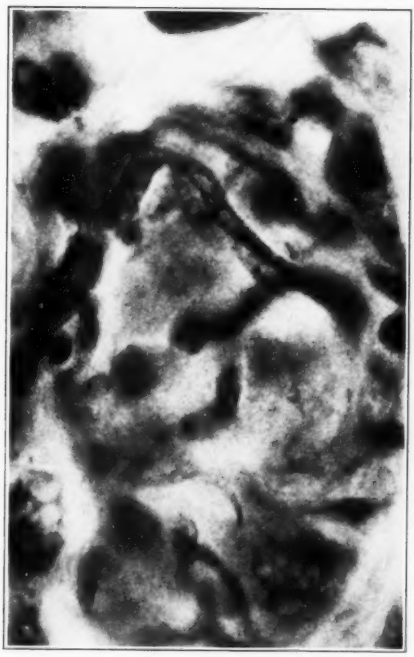

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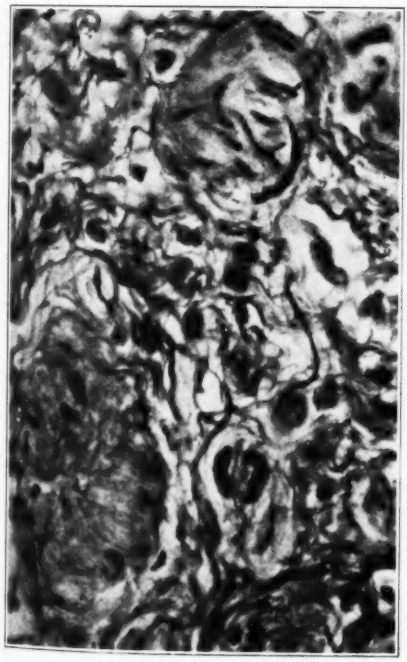

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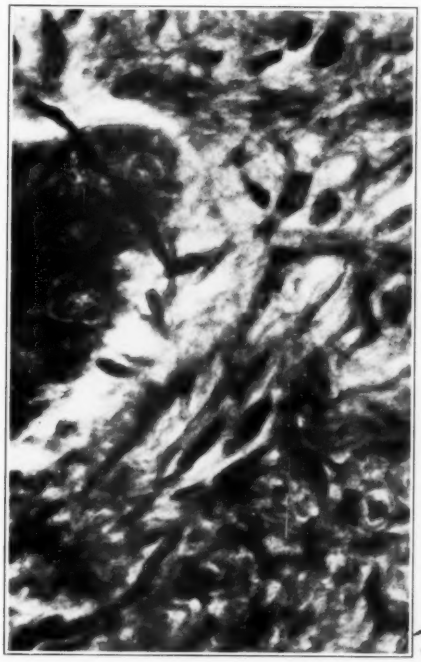

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Histology of Melanoma. II

PLATE 55

FIG. 5. Field from a small neuroma, to demonstrate the coarse medullated fibers of which it is largely composed, together with some finer fibrils. This was chiefly made up of fibers like those at the upper right and lower middle portion of the picture. Distortion of the large fibers is due to formalin fixation.

FIG. 6. A nerve trunk lying in a tumor nest; note the perineural sheath.

FIG. 7. A trunk with coarser fibrils impinging upon the base of a tumor nest, but not penetrating into it very deeply.

FIG. 8. A similar trunk leading into a small tumor alveolus; here, too, the fibrils appear to fail to penetrate deeply; one of them has a lancet-shaped terminal swelling, indicating that the fibrils do not communicate or connect with those among the cells.

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Histology of Melanoma. II

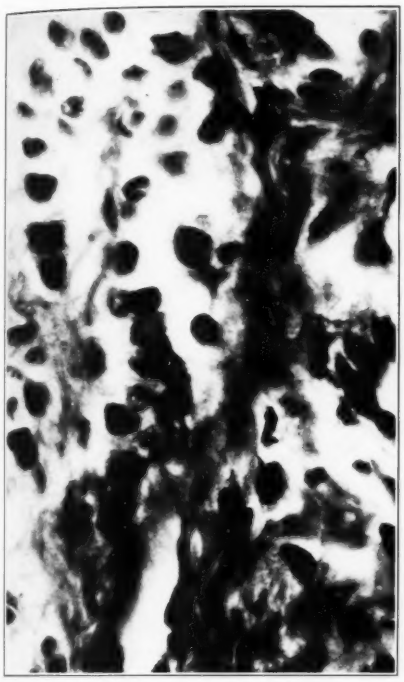
PLATE 56

FIG. 9. Coarse nerve bundle from a raised, sessile mole. It runs in the interalveolar stroma and apparently terminates in finger-like branches.

FIG. 10. Compact nerve fibrils, with small vacuoles, seen in the tumor tissue of a sessile, non-pigmented mole of the face.

FIG. 11. A "lame foliacée" from the tumor shown in Fig. 10, to be compared with Figs. 1, 2 and 3. A few structures resembling rudimentary Dogiel terminals may be seen and a body very much like an enormously elongated nucleus, but probably a nerve terminal, may be noted near the center of the field.

FIG. 12. Erector pili smooth muscle fibers intimately associated with the tumor shown in the preceding figures, coarse "nevus fibers" and nerve fibers are seen here and there.



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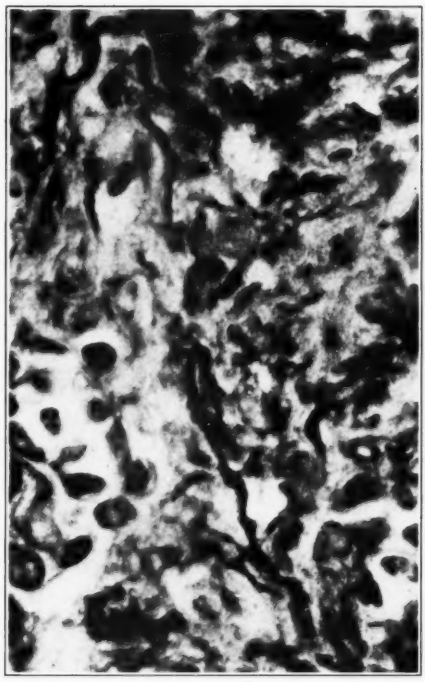

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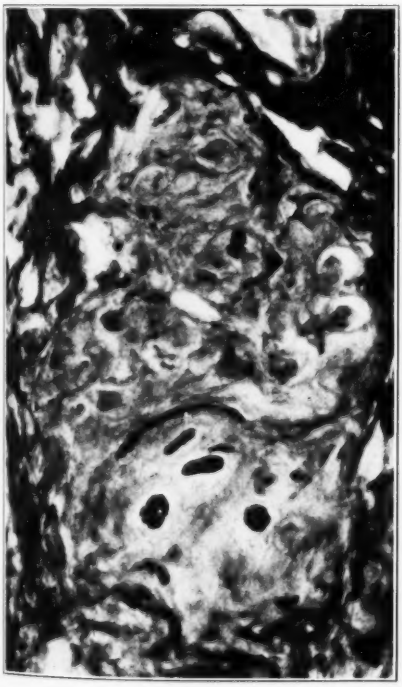

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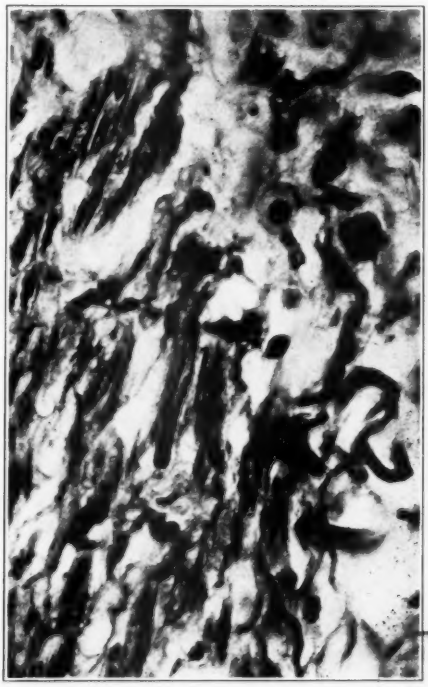

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Histology of Melanoma. II

HISTOCHEMICAL STUDIES BY MICROINCINERATION OF NORMAL AND NEOPLASTIC TISSUES *

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AND
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Policard and Doubrow [1] in 1924 were the first investigators to apply the technique of microincineration to a comparative study of normal and malignant tissues. Apart from finding a slight difference in the mineral ash content between the cancerous and corresponding normal cells, they demonstrated most convincingly the advantages of this method, not only as a histochemical but also as a pathological technique. Recently Scott [2] devised an improved method by which the inorganic structure of the incinerated material can be more readily observed, and also by which the intensity of the mineral salt deposits is more appreciably recognized. With the recent use of this method the results obtained by Horning and Scott,[3] which indicate that morphogenesis in the developing embryo is accompanied by apparent differentiation of the inorganic constituents, are extremely interesting when correlated with the Cohnheim "embryonal theory," according to which tumors are held to proliferate in much the same way that embryonic tissue grows. Under these circumstances it was considered advisable to reinvestigate the inorganic nature of malignant and normal tissues by means of this improved technique. The neoplastic material consisted of human medullary duct carcinomas of the breasts, as well as of several of the scirrhous types,† together with the following transplantable mice tumors: M 63, S 37 and S 180.

* Aided by an appropriation from a grant made by the Rockefeller Foundation to Washington University for research in science.

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† We desire to express our thanks to Dr. Robert Elman who supplied us with this material. Thanks are also due to Dr. Leo Loeb and Dr. F. Carter Wood for providing these rodent transplanted tumors.

The inorganic remains of the various cancerous growths were studied in dark-field illumination obtained by using a Zeiss cardioid condenser. The material was incinerated in an electric quartz oven at a temperature of 650° C in the manner previously described.[4] Comparative examinations of all incinerated neoplastic material, with the accompanying control histological preparations, demonstrated how admirably the cancerous newgrowths lend themselves to this technique, as the fixed mineral elements retain their distinctive morphological organization.

The first preliminary observations of the microincinerated tumors confirmed the previous contentions of Policard and Doubrow that cancerous tissue remains carbonized longer than normal tissues. Another striking phenomenon we detected was that the intensity and distribution of the mineral ash deposit is more abundant within the invading newgrowths than in the surrounding stroma. This extraordinary concentration of the inorganic salts in the tumor tissue is well shown in the accompanying photomicrographs (Figs. 1, 3 and 4), and was found to be a feature peculiar to both human and rodent neoplasms.

More detailed analysis of the breast carcinomas shows that the infiltrating cancerous newgrowths are characterized by a heavy deposition of mineral salts when viewed at low magnifications. Observation with oil immersion lenses shows that the nuclei contain rather more ash residue than do nuclei of the normal duct tissue. This deposit is concentrated along the peripheral margins of the nuclei (Figs. 3 and 4) and corresponds well with the hyperchromatization described by Horning and Richardson [5] in malignant growths. The nuclear inorganic salts contain visibly more iron oxide than do those of normal cells.

The remaining mineral deposits in the cytoplasm are more abundant than in the normal cells and contain an appreciable quantity of calcium and iron salts.

There are evidently at least three factors causing an increased appearance of ash in the cancerous ingrowths when viewed with the low power of the microscope. There are more nuclei per unit area present than in the adjacent fibrous stromal tissue (Figs. 3 and 4), and the nuclei themselves contain more inorganic residue than do those of the normal cells (Figs. 2 and 4). In addition to these factors the cytoplasm of the neoplastic cells contains more mineral salts than is usual for such tissue (Fig. 2).

A comparative examination of the transplantable rodent carcinoma and the sarcomatous growths reveals that both are rich in mineral ash deposit. The differences, however, between the stroma and the infiltrating malignant growth are less marked than in the human neoplasms, as the cells composing the surrounding stromal regions contain more inorganic residue. Nevertheless, the distinction between the pathological and the adjoining healthy tissues is clearly defined. Another interesting feature is that the nuclei of the sarcoma cells of the mice appear to contain greater concentrations of inorganic material than do the nuclei of carcinoma M 63, while the mineral salts in the cytoplasm are distributed in a more diffuse manner.

A survey of the inorganic structure of neoplastic and normal tissues demonstrates conclusively that malignant growths are richer in their mineral contents than normal tissues — especially in calcium and iron oxide. Beebe and Clowes,[6] employing biochemical methods, have shown that necrotic tumors contain more calcium oxide than rapidly growing cancers free from necrosis. Although necrotic areas incinerate less readily, our observations by microincineration yield supplementary evidence.

Conclusions

The results obtained from this investigation are of interest, inasmuch as they have demonstrated that functional differences between cancer and normal tissues are exhibited inorganically by marked variations in their inorganic content.

An additional feature is the close similarity between developing embryonic cells and cancer cells — a similarity which is mainly due to the distribution and arrangement of mineral salts. Both of these cells are characterized by an extraordinary variation in the intensity, concentration and orientation of their inorganic constituents, and contrast greatly, on the other hand, with the appearance of the mineral elements in the healthy adult tissue, which remain proportionally fixed. This "inorganic reversion" of the cancer cell, as revealed by microincineration, is interesting in view of Cohnheim's theory to the effect that malignancy depends upon the retention of small groups of cells of embryonal character.

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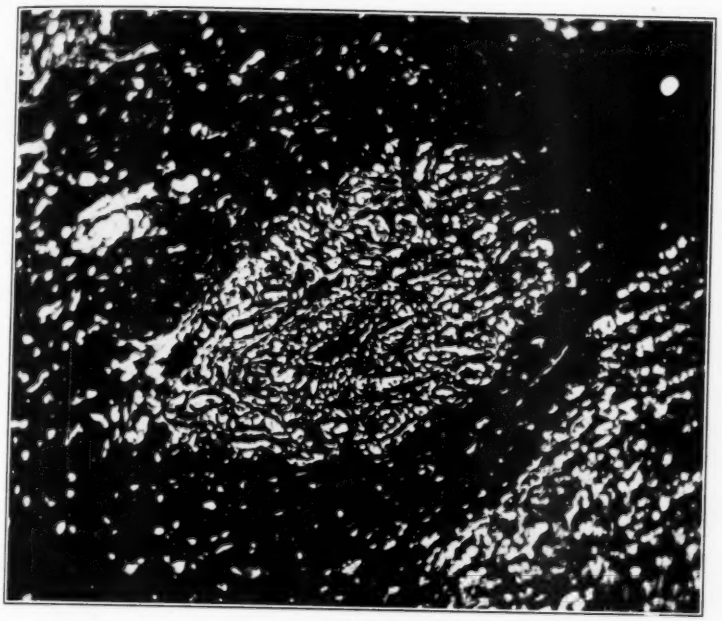
DESCRIPTION OF PLATES

PLATE 57

FIG. 1. Photomicrograph of an incinerated section through a human duct carcinoma of the breast. Observe the difference between the mineral ash content in the infiltrating malignant tissue and that of the adjacent fibrous stromal structures.

FIG. 2. Showing camera lucida drawing of the same, as seen under a higher magnification. Note the conspicuous peripheral accumulation of inorganic salts in the nuclei of the invading tumor tissue.



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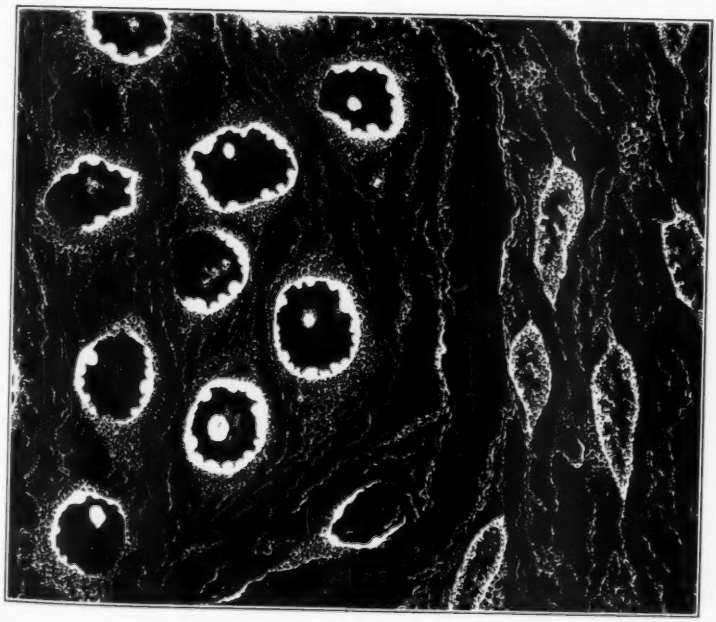

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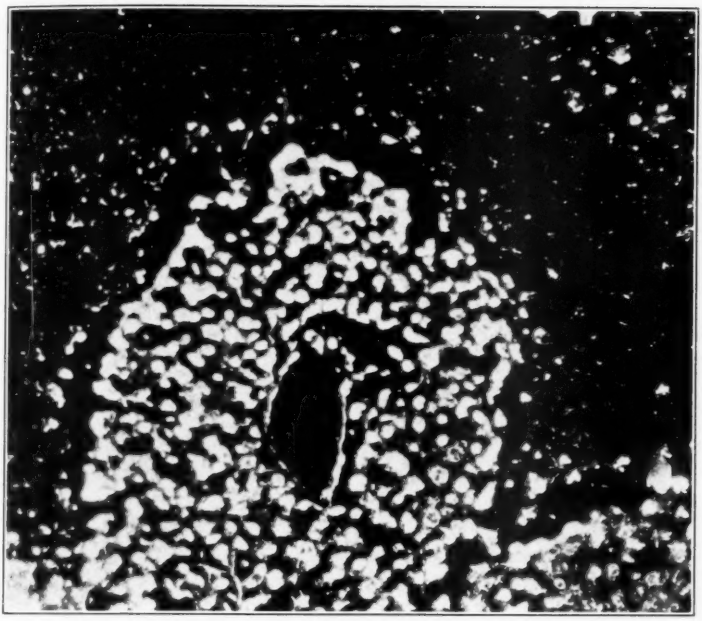
PLATE 58

FIG. 3. Photomicrograph taken with higher magnification, depicting incinerated sections of a human duct carcinoma of the breast. Observe the increased mineral salts in the invading growths and compare this with the inorganic remains of the surrounding stroma.

FIG. 4. Showing the curious peripheral concentration of mineral salts in the nuclei of the neoplastic tissue.



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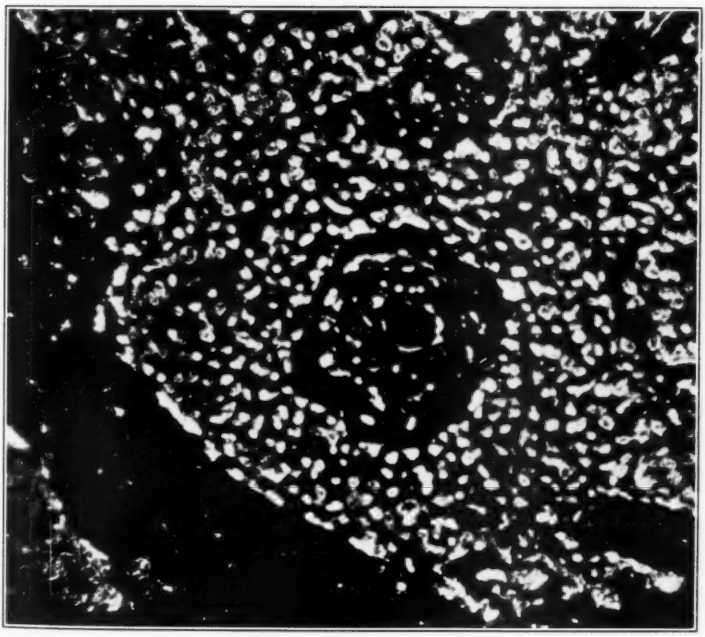

4

A CASE OF MULTIPLE PAPILLOMATA OF THE LARYNX WITH AERIAL METASTASES TO LUNGS *

HENRY B. HITZ, M.D., AND ERNST OESTERLIN, M.D.
(*From the Milwaukee Hospital, Milwaukee, Wis.*)

Dorothy G., aged 2 years, white, female, was admitted to the Milwaukee Hospital, March 16, 1925, with difficulty in breathing and inability to speak aloud. The history, as obtained from her parents, was that at the age of 1 year she had a dry irritative cough followed by a gradual loss of voice, and since that time has been able only to whisper. For two months prior to her admission to the hospital she had occasionally at night a rather marked dyspnea. The child had been a full-term baby, normally delivered, was breast fed for the first thirteen months, and had previously not been ill. At the time of admission she was well developed and well nourished. There was nothing abnormal in the family history. The father and mother were strong and physically well, as were two other children, one older and one younger.

Examination at the time of admission showed no abnormality in the nose, nasopharynx or throat. The tonsils were small and normal in appearance and there was no perceptible adenoid mass present. The larynx showed what appeared to be multiple papillomata covering the vocal cords and much of the ventricular bands.

On the following day, March 17, under direct laryngoscopy, numerous papillomatous masses were removed from the larynx and sent to the laboratory for sectioning. The pathological diagnosis was benign papilloma.

After a short period of time in the hospital she was discharged, but was readmitted with similar symptoms, Sept. 9, 1925. Examination showed redevelopment of papillomata in the larynx, and they were removed again under direct laryngoscopy. She was discharged with improved breathing and speech, but a few months later, on March 20, 1926, she was readmitted and underwent similar treatment. On this occasion, while still in the suspension apparatus, she was given an application of radium for two and one-half milligram hours, and after a few days discharged.

* Received for publication December 8, 1931.

The patient was readmitted to the hospital on June 7, 1926, and on the 19th, removal of the growth was done again with a similar application of radium. She was retained in the hospital under constant observation, but on August 17 she developed such marked stenosis that a tracheotomy was performed and the tube retained until her subsequent death.

Following the tracheotomy, at several intervals, large masses of papillomatous material were removed from the larynx. However, her physical condition was so improved by the tracheal operation that her father insisted upon taking her home. In spite of instructions that he was to bring her back at stated intervals for the cleaning of her tracheal tube, this was but rarely done.

In March 1927, the last removal of small papillomatous masses from the larynx was done. There seemed to have been a marked reduction in the general tendency to redevelopment. However, the following summer she was brought into the hospital in a dying condition by her father, he having attempted to change the tracheal tube and failing to reinsert it properly. In all, this child was in the suspension apparatus ten times.

An autopsy was performed and the following report of Dr. Oesterlin, describing unusual pathological findings, is here presented.

Gross Findings

Well developed, normal child, 3 years of age, with a tracheotomy wound and tube *in situ*. After removal of the cannula, a large papillary tumor was found projecting into the wound.

Upon opening the trachea, the whole of the larynx and the upper part of the trachea were found to be filled with cauliflower-like masses, the single elements of which presented a distinct papillary structure varying in size from that of a millet seed to that of a lentil. A few of these masses were larger and attained the size of peas. They were grayish white in color, and of firm consistence.

The larynx was entirely filled with these masses in such a way that no details of its structure could be made out. The epiglottis could hardly be seen and was almost entirely covered with papillary tumors.

The adjacent organs, the esophagus and the large blood vessels, were intact and there was no invasion of the tumor into them. The thyroid gland was small and showed no lesions.

The lower half of the trachea and the large bronchi showed no pathological findings. In the lungs, however, there were many small cavities, varying in size, some as large as hazel nuts. Frequently their connection with the smaller bronchioli could be traced. The walls of these cavities were covered with fine granules about the size of millet seeds. The other organs throughout the body were found to be normal.

Microscopic Findings

The primary growth from the larynx and trachea (Fig. 1) presents a stalk of connective tissue, rich in hyperemic blood vessels. The epithelial lining of this stalk consists of many layers of squamous stratified epithelial cells without hornification. In the upper layers the cells are large and polyhedral. In the basal layer the cells are columnar and their nuclei stain more intensely with hematoxylin. The cells are regular throughout, without any remarkable difference in size or shape. There are a few polymorphonuclear leukocytes scattered among the epithelial cells. The submucosa is in some areas invaded by lymphocytes. As is usual in papilloma of the larynx there are many mitoses.

The nodules in the lungs (Fig. 2) sometimes form compact masses, but frequently contain central lumina which are either empty or filled with desquamated cells and polymorphonuclear leukocytes.

The tumor cell strands consist of squamous, stratified epithelial cells resembling those found in the larynx. There are large, polyhedral cells which stain lightly with hematoxylin. Columnar cells are frequent; they form not only the basal layers as in the primary growth, but are also to be found everywhere between the polyhedral cells. Mitotic figures are still more frequent than in the tissue from the larynx.

The strands of epithelial cells are in some areas directly adjoining the lung alveoli; in others there is a zone of connective tissue which separates the tumor cells from the walls of the alveoli.

A view of Fig. 3 shows a white space lined by columnar epithelium, which apparently corresponds to the epithelial lining of a bronchiolus. This lumen contains, besides detritus and white blood cells, some squamous stratified epithelial cells of exactly the same structure as were found in the papilloma of the larynx (Fig. 4).

Comment

The interpretation of the primary growth is not difficult. There is little doubt that here we were dealing with multiple papillomata of the larynx. The growths were removed from different sites at different times and always showed the same type of growth as can be seen in Fig. 1.

The presence of these tumor masses in the lungs is not as easily understood. At first glance, on the postmortem table, it was thought they might be complicating tuberculosis, but under the microscope they are distinctly seen to be a neoplastic growth (Fig. 2). The question then arises whether or not these nodules in the lungs were metastases from the tumors in the larynx. The age of the patient, and the evidently benign type of growth, seem to speak against metastases. In order to detect and definitely rule out a metastatic dissemination of the tumor through lymph channels, many sections of the peritracheal and peribronchial glands were made without finding metastases in these lymphatic structures.

Another possibility is that the nodules in the lung had an origin analogous to the growth in the larynx, resulting in a condition of multiple papillomata in the bronchioli. In this case it must first be assumed that a metaplasia of the columnar epithelium of the bronchiolus took place into a squamous stratified epithelium, from which this type of tumor only can arise, but no reason was found to substantiate such a change. Furthermore it is difficult to understand why the lower half of the trachea and all of the larger bronchi should have been free and only the smaller bronchioli filled with tumor masses. It is also not easy to trace the connection of each nodule with the bronchiolus; sometimes the tumors were lying free in the lung alveoli.

The interesting findings in Figs. 3 and 4 suggest another interpretation. These slides show plainly how the tumor completely fills a part of the lumen of a bronchiolus. This would suggest the interpretation that all of these tumors in the lung had the same common origin, namely, that the tumor masses growing too rapidly in the larynx were detached and carried into the bronchi by aspiration. They passed the larger bronchi but were caught in the bronchioli, obstructing their lumina. In this way they became implantation metastases and began to grow into the alveoli. In some areas con-

nective tissue had already been formed, apparently as a reaction against the foreign body, such as would result from stray particles of tumor tissue invading the lung.

Discussion

The text-books of pathology mention little or nothing about metastases through the bronchi. Only in the French literature is attention given to this possibility. Ribadeau-Dumas [1] mentions the "greffe bronchique" (bronchial graft) for aspirated particles of a cancer of the esophagus which become grafted in the parenchyma of the lung.

Letulle [2] describes them as uncommon, but very characteristic. He mentions as primary growths, epitheliomata of the pharynx, larynx and trachea. It is easily understood that pedunculated vegetations in the larynx may at intervals shed some fragments or isolated elements, which are still endowed with karyokinetic activity.

Letulle and Jacquelin [3] have described a very interesting case of a collapsed lung in which a primary cancer developed. "From this primary growth neoplastic colonies arose in the normal bronchioli by 'aspiration.'" They were grafted and formed carcinomatous nodules. "Around the bronchus they grew, not only into the interior of the bronchiolus, but especially into the alveoli connected with this bronchiolus. This almost systematic disposition of the peribronchic alveoli by the cancer cells cuts out a circular, almost regular zone." Letulle has coined for this type of metastases the term, "metastases aeriennes" (aerial metastases).

In the case here presented, the tumor was histologically non-malignant, both in the larynx and in the lung. Nevertheless the tumor represented an actively growing, benign neoplasm, from which small fragments frequently became detached. It was possible to find these fragments in the center of a bronchiolus and to trace the origin of the tumor nodules in the alveoli from the invaded bronchioli. In spite of a careful search, no invasion of the lymphatics by tumor cells was observed.

All of these facts give definite evidence of the invasion of the lungs, through the bronchi, by a benign papilloma, primary in the larynx. If this be true, closer attention should be paid to the occurrence of this type of metastasis, with the probability that more cases of this type will be observed.

SUMMARY

The case here described is one of multiple papillomata of the larynx with metastases to the lungs through the bronchi (aerial metastases).

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DESCRIPTION OF PLATE

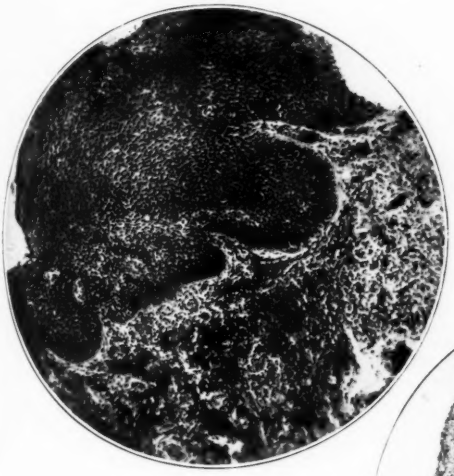
PLATE 59

FIG. 1. Primary growth. Papilloma of larynx.

FIG. 2. Aerial metastases. Low power.

FIG. 3. Bronchus showing aspirated tumor mass in its lumen.

FIG. 4. Central part of lumen (papilloma under high power).



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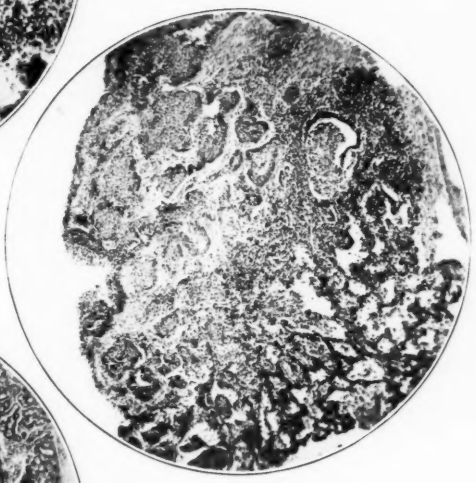

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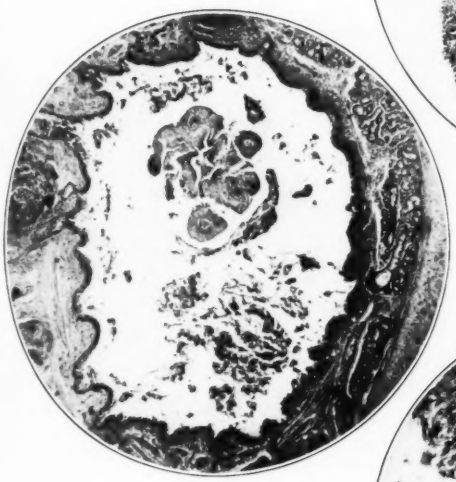

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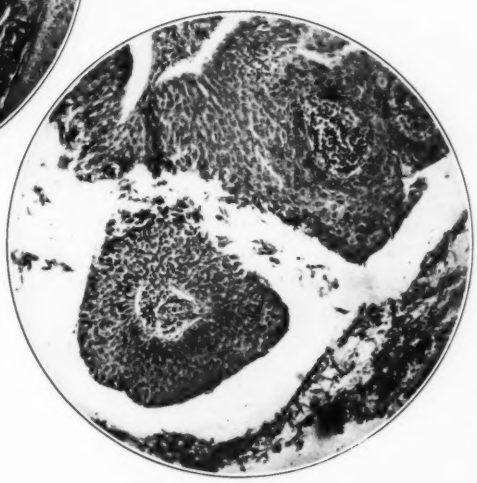

4

Hitz and Oesterlin

Multiple Papillomata of Larynx

LUMBOSACRAL TERATOMA ASSOCIATED WITH SPINA BIFIDA OCCULTA *

REPORT OF A CASE WITH REVIEW OF THE LITERATURE

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Mixed tumors and teratomas of the sacral and sacrococcygeal regions have long been recognized and very frequently described in the literature. Ewing [1] reports: "They are bulky masses, present at birth, lying on the dorsal surface of the sacrum and coccyx, and adherent to or enclosed within the periosteum, or connected to the bone by a pedicle. Others lie anterior to the sacrum, connected with this bone, or with the rectum, and projecting into the pelvis. The structure presents cystic and solid portions similar to those of teratoid tumors, including cysts lined by various types of epithelium, dermoids, segments of intestinal mucosa, gland structures, fat, muscle, cartilage, and bone and finally, portions of nervous and glia tissue. In addition, a great variety of rudimentary organs are observed. These include segments of intestine with mesentery, rudimentary esophagus, stomach, and buccal cavity with salivary glands, pulmonary parenchyma, bronchi with cartilaginous rings, thyroid, pancreas, spleen, adrenal, kidney, brain with ventricles and choroid plexus. The bones may reproduce well-formed extremities, as forearm and hand, tibia, femur, and joint, pelvis and extremities, toes, and eyes. In fact, Askanazy regards the sacral teratomas as the most prolific in the production of rudimentary organs." On the other hand similar lesions of the lumbosacral region are exceedingly rare, and for this reason we present a case of lumbosacral teratoma associated with a lower lumbar and sacral spina bifida.

CASE REPORT

CLINICAL HISTORY: *Female, aged 15 months, mass in lumbosacral region since birth. Spina bifida. Unexplained leukocytosis. Extirpation of teratomatous tumor. Recovery.*

E. Del M., a Mexican female, aged 15 months, was admitted to the Bobs Roberts Memorial Hospital for Children on August 3, 1931. According to the

* Presented before the Chicago Pathological Society, February 8, 1932.
Received for publication February 29, 1932.

father the child was born at term, and was apparently a normal baby except for a lump over the lower part of the back. There was no information available as to how large the mass was at birth, but the parents believed that it had gradually increased in size. There had been no other complaints. The child talked when 12 months' old and walked at 14 months. At times she indicated when she wished to micturate or defecate.

Examination: The child was very well developed and weighed 9420 gm. (20.7 pounds). There were palpable cervical glands. The head measured 47 cm., and the chest 47 cm. in circumference. The child walked without difficulty, and seemed quite normal except for the large, soft, tumor mass in the lumbosacral region (Fig. 1). The mass measured 10.5 by 9 by 4 cm., and was covered by healthy skin, upon the surface of which were two small, pedunculated nodules. There was no detectable abnormality of sensation. Reflexes were all present and equal.

X-ray Report: Roentgenograms of the entire vertebral column from the first thoracic vertebra downward revealed a marked defect of the posterior part of the neural arches of the fourth and fifth lumbar vertebrae and of the entire sacrum.

Laboratory Data: Examination of the urine revealed nothing abnormal. The blood Wassermann reaction and the Kahn test were negative. Numerous examinations of the blood revealed a leukocytosis of from 11,850 to 27,000. The percentage of polymorphonuclear (neutrophilic) leukocytes varied from 15 to 35, while mononuclear leukocytes ranged from 59 to 80 per cent. Definitely pathological cells could never be detected although many smears were examined. The red blood cells varied from 3,680,000 to 4,600,000 per cmm. The hemoglobin varied from 65 to 88 per cent by the Sahli method.

Operation: On August 6, 1931, the tumor was excised. A midline incision was made over the mass through the skin and a very thick layer of adipose tissue down to a small tumor nodule about 2 cm. in diameter. The adipose tissue was readily dissected off the mass, revealing a narrow stalk passing downward from the nodule through the defect in the posterior vertebral arch into the spinal canal. The stalk was cut through at its proximal end. The exact relation of the stalk to the spinal meninges and neural tissue was not determined. No operative repair of the spina bifida was attempted.

Postoperative Course: Following the operation the patient made an uneventful recovery and was discharged on September 13, 1931. Examination before discharge from the hospital did not reveal any abnormal, objective neurological findings. The etiology of the leukocytosis was never determined. Roentgenograms were made of all long bones, the chest and the gastro-intestinal tract; none of which revealed anything abnormal. The child was last seen on October 13, 1931, at which time she seemed in excellent health. A leukocytosis of 13,100 was still present. The red blood cells numbered 3,820,000 per cmm. and the hemoglobin was 76 per cent. The head has not enlarged.

Neoplasm: The tumor itself was fixed in formalin, embedded in paraffin, and stained with hematoxylin-eosin, mucicarmine, Van Gieson's acid picro-fuchsin, and Freeman's method for nerve fibers. On incising the tumor mass a small cyst, the wall of which was pedunculated and thrown into folds, was opened (Fig. 7). This cyst contained a thick, glary, tenacious, mucus-like fluid.

Microscopic Findings

Fluid: The mucus-like fluid was smeared immediately after removal, fixed with alcohol and stained with hematoxylin and eosin (Fig. 3). It contained numerous cells which varied greatly in size from small cells with very dark staining, round nuclei, and a small amount of granular, eosinophilic cytoplasm, to enormous cells with nuclei as large as the entire area of the smaller cells. The nuclei contained a heavy chromatin network. The cytoplasm of the large cells was granular, slightly eosinophilic and often markedly vacuolated so as to give the appearance of foam cells. The larger cells were often multinucleated, and contained from two to four nuclei. They also tended to group together in rows of two to five cells. Dr. William Bloom of our Department of Anatomy examined this smear very carefully, and was of the opinion that the larger cells were macrophages. He also thought that all stages of transition from lymphocytes to macrophages were demonstrable. An occasional polymorphonuclear leukocyte was also present. There were no ciliated cells in the fluid, as was noted in Kubie and Fulton's case.[2]

Cyst Wall: The cyst was lined with a columnar epithelium a single cell in thickness in most places; however, at points, it assumed a pseudostratified appearance (Fig. 7). The cells were moderately tall columnar in type with basal, oval-shaped nuclei. There was a definite basement membrane. The external surface of the cells was covered with numerous long cilia (Fig. 5). There was no evidence of the brush border which is typical of intestinal epithelium. Interspersed among these columnar cells were many typical, large, swollen goblet cells (Fig. 2). In sections stained with mucicarmine the contents of the goblet cells and the material found in the lumen of the cyst stained bright red. Underlying the epithelium and occupying the core of the papillae there was a very vascular, loose connective tissue. In this connective tissue layer were to be found many glandular structures. These structures were composed of a collection of alveoli lined with a single layer of columnar cells and many goblet cells. These glands frequently lay beyond this loose connective tissue in the underlying layer which was a thick band of smooth muscle, or even in the layer of connective tissue which blended with the overlying adipose tissue, or with the remainder of the tumor.

The remainder of the tumor, *i. e.*, that part ventral to the cyst, was composed of a rather loose connective tissue framework in

which there were many bundles of myelinated nerve fibers, a ganglion containing many ganglion cells, an atypical Vater-pacinian corpuscle, a lymph node and much smooth muscle. The myelinated fibers did not differ from similar bundles of fibers found elsewhere in the body (Fig. 6). The ganglion (Fig. 6) was comparable to those found normally in the sympathetic nervous system or to the posterior root ganglia. It contained many typical large ganglion cells. Unfortunately the various stains did not demonstrate the processes of these cells and it was impossible to state whether they were unipolar or multipolar cells. Each was surrounded by a group of typical spindle-shaped capsule cells and numerous nerve fibers could be seen running through the ganglion. The Vater-pacinian corpuscle (Fig. 4) was found in the tumor not far from the ganglion and amongst the bundles of myelinated nerve fibers. The corpuscle had a definite connective tissue capsule, beneath which were several layers of fine concentric rings associated with a few flat, elongated nuclei. This large ring of fibers enclosed five smaller but similar rings, the central portions of which contained numerous concentric rings of fine fibers but no nuclei. In one of these smaller rings a central canal comparable to the inner bulb of the normal corpuscle was seen. There was also within the larger ring a small collection of cells with large oval nuclei and no definite cytoplasm. The lymph node presented nothing remarkable. It was composed of numerous small, round nuclei without definite cytoplasm. They were rather heavily stippled with chromatin. There was a definite connective tissue framework and a few small blood vessels.

Discussion

Dr. George W. Bartelmez of our Department of Anatomy very kindly examined the sections of this tumor. He was of the opinion that the ciliated columnar epithelium which lined the cystic cavity was most comparable to the epithelium of the respiratory system. The glands which were definitely mucous in character were comparable to the tracheal glands. Admittedly other possibilities must be considered. It would seem obvious that any relation between this tissue and ependyma can be ruled out definitely for several reasons: the epithelium was definitely a mucus-secreting structure as it contained numerous, large goblet cells, and the cells and lumen

of the cyst contained material which stained well with mucicarmine; also, there was a clear-cut, limiting membrane to the epithelial layer; and further, the mucous glands in the deeper structures did not correspond with any structure related to ependyma. The possibility that this is comparable to intestinal epithelium must also be considered; however, the absence of the typical brush border is against that possibility. The epithelium, although not greatly different from that of the genital tract, contained numerous goblet cells which are seen in the cervix, but not found in the Fallopian tube or in the body of the uterus. The cells in the neoplasm, however, are not as tall as those seen in the cervix or in the glands of the cervix.

With the association of this mucous epithelium, the smooth muscle, connective tissue and lymphoid tissue, the myelinated nerve fibers, the Vater-pacinian corpuscle and the ganglion, we obviously are dealing with a trigerminal congenital neoplasm — a teratoma.

Such structures, as a diligent search through the literature since 1800 revealed, are exceedingly rare in the lumbosacral region. Teratomas in the region of the coccyx, anterior to the sacrum, even connected with the neural structures in the spinal canal through a defect in the anterior portion of the sacrum, are relatively common. It would appear that these develop from remaining vestiges of the neurenteric canal and postanal gut. However, no such structure is so situated as to explain the origin of this tumor, or of the obviously closely related teratomatous spinal cord cysts of Kubie and Fulton,[2] and the intradural teratomatous tumor of the spinal cord reported by Hosoi.[3] The first reported case similar to the authors' is that of Sonntag [4] in 1925. His patient was a male child 4 months' old, with a midline lumbosacral tumor. The illustration of his patient is almost identical with Fig. 2. The tumor at operation was found to hang on a stalk as thick as a child's finger, which disappeared through a fascia-muscle-bone defect to attach to the dura. Telangectasia, fibrous tissue, fatty tissue and cartilage were described in the tumor, but detailed histological study was not reported.

The second case was Aloi's [5] patient — a female, aged 19 years, who had had a lumbosacral tumor associated with spina bifida since birth. It had been considered inoperable at birth, and in about a year the tumor reached the size of a pigeon's egg. It ruptured spontaneously and drained clear, watery fluid. It then drained sero-

sanguineous fluid intermittently until the time of admission. There were no abnormal neurological findings. At operation an apple-sized, soft, elastic, sessile tumor was removed and the pedicle ligated as it left the sac. The underlying spinal canal defect was closed by osteoplastic resection. Recovery was practically uneventful. Histological examination proved the lesion contained atypical and undifferentiated muscle fibers, convoluted glands resembling those of the large intestine more than the small, mucous glands, as proved by staining methods, lymphatic tissue and nerve filaments.

It would seem very likely, therefore, that we were dealing in the case reported here with a trigerminal, congenital neoplasm — a teratoma — a "twin" which had not gone on to full development. Such a division of the ovum, so as to produce two organisms, might have occurred at any time during the presence of the primitive streak, *i. e.*, up to the fourth week following fertilization (Bartelmez [6]), and would have been due to the physiological isolation of the two halves of the primitive streak.

Summary

1. A case of a dorsal lumbosacral teratoma in a Mexican female of 15 months is reported. Two additional cases have been collected from a review of the literature. All of the cases have been associated with spina bifida of the lower lumbar region. None of the three has had neurological abnormalities.

2. The tumor in the authors' case is composed of a mucus-containing cyst lined with ciliated, columnar epithelium thought to be comparable to respiratory epithelium, smooth muscle, connective tissue, bundles of myelinated nerve fibers, a Vater-pacinian corpuscle, a ganglion containing typical dorsal root or sympathetic ganglion cells, and lymphoid tissue.

3. The teratoma is thought to be an undeveloped "twin" due to division of the primitive streak during the first four weeks of embryonic life.

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DESCRIPTION OF PLATES

PLATE 60

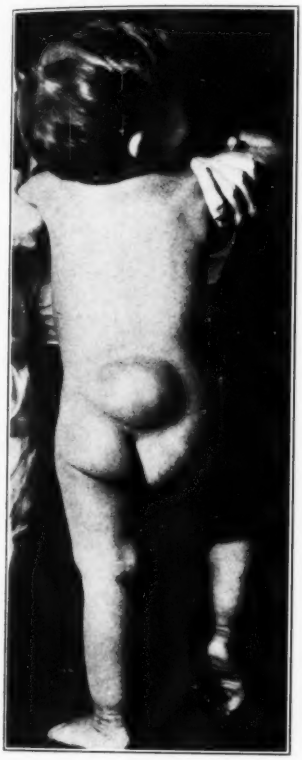
FIG. 1. 15 months' old Mexican child with a teratoma in the lumbosacral region.

FIG. 2. Portion of cyst wall containing many goblet cells. Hematoxylin and eosin. × 200.

FIG. 3. Smear of the fluid from the cyst showing cells of various sizes including one large vacuolated cell (foam cell) in the upper right-hand corner. Hematoxylin and eosin. × 200.

FIG. 4. An atypical Vater-pacinian corpuscle. In the lower portion is a group of concentric rings enclosing a clear space, the inner bulb. Hematoxylin and eosin. × 200.

FIG. 5. Section of the cyst wall showing mass of cilia. The epithelium at this point is pseudostratified. Hematoxylin and eosin. × 1200.



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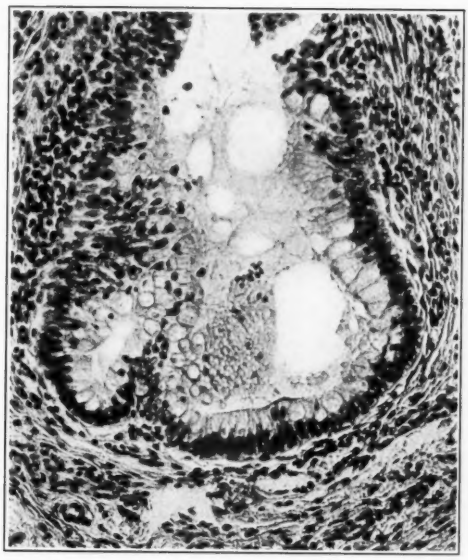

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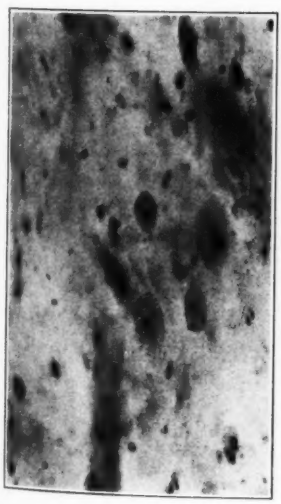

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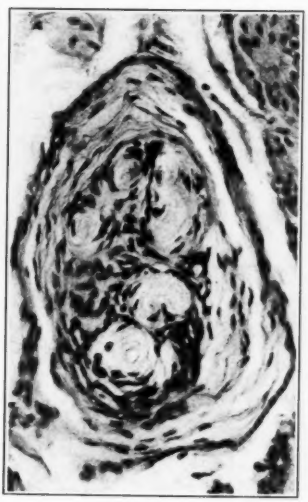

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5

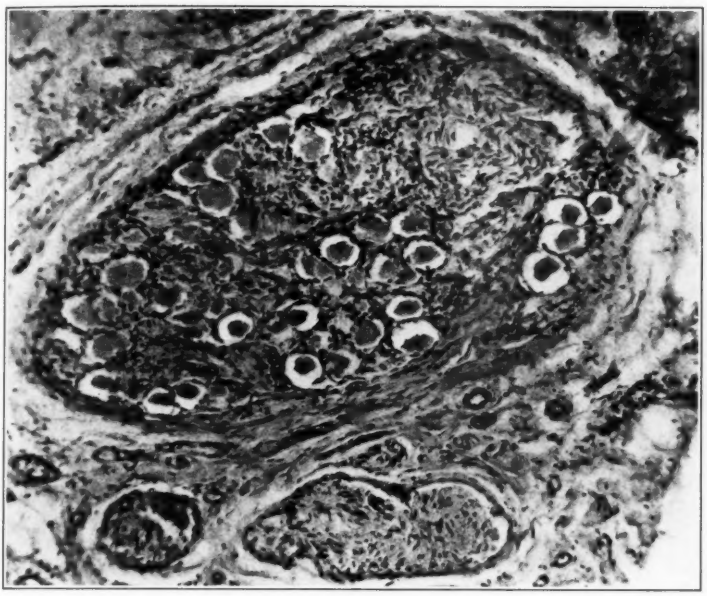
Bucy and Haymond

Lumbosacral Teratoma

PLATE 61

FIG. 6. Ganglion containing numerous ganglion cells, each surrounded by several capsular cells. The two bundles of myelinated nerve fibers are present in the lower part of the illustration. Hematoxylin and eosin. × 100.

FIG. 7. Portion of cyst wall lined by simple columnar and pseudostratified columnar epithelium. There is a group of goblet cells in the upper left-hand corner. Hematoxylin and eosin. × 150.



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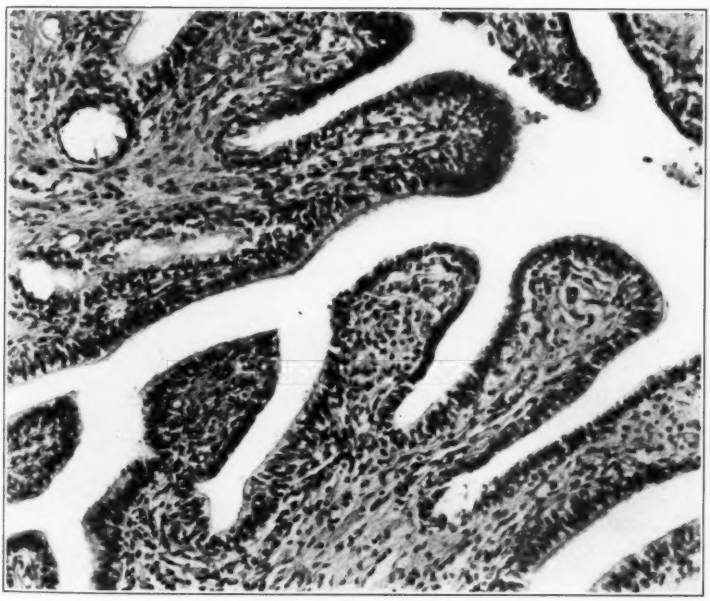

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Bucy and Haymond Lumbosacral Teratoma

MICROCOCCUS PHARYNGIS SICCUS ENDOCARDITIS *

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The following case of acute vegetative endocarditis is reported in order to call attention to an instance of a fatal bacterial infection in man caused by a microörganism ordinarily considered non-pathogenic for man, namely, *Micrococcus pharyngis siccus*. It was first identified by Von Lingelsheim. He found it to be a common inhabitant of upper respiratory passages of normal individuals. It was included by Bergey [1] in the Gram-negative cocci of the genus Neisseria and named the *Neisseria sicca*.

Schultz [2] in 1919 recorded the first case that we have been able to find of bacterial infection in man ascribed to this microörganism. His was a case of acute vegetative endocarditis with multiple secondary foci of involvement in a previously healthy young adult man. In his case the microörganisms were recovered from the blood stream in pure culture twice during life, and from the vegetations and the spleen at autopsy. It is to be noted that the heart at postmortem examination showed no evidence of preëxisting disease.

CASE REPORT

Clinical History: A. C., a doorman by occupation, 27 years of age, unmarried, born in this country, entered Bellevue Hospital, May 25, 1930, for the third time in five years. He had been a regular attendant of the Adult Cardiac Clinic of Bellevue Hospital since 1925, when his symptoms of heart disease became manifest.

At the age of 17 he had a mild attack of rheumatic fever associated with pneumonia and pleurisy. A severe attack of rheumatic fever lasting five months occurred at the age of 22. Between the first and second attacks he had had frequent joint pains. In 1925, following the second attack, he suffered his first heart failure and was admitted to the hospital with signs and symptoms of carditis and congestive heart failure. He recovered after a month and then attended the cardiac clinic. There he was given maintenance doses of digitalis which he continued to take thereafter. He was then able to do light work until July, 1929. At that time he had a recurrence of carditis and congestive heart failure. After seven weeks he was discharged from the hospital, improved. He continued to attend the cardiac clinic and to work as a doorman at a theater.

* Received for publication February 11, 1932.

He also continued to take digitalis in maintenance doses, and remained comfortable until May 11, 1930.

On that day, following a meal, he experienced cramp-like pain in the lower abdomen, soon followed by retching and vomiting. He reported to the cardiac clinic on May 16. At the clinic his temperature was noted as 102.5° F and he appeared acutely ill. He went home to bed, noticed marked weakness, gastric distress, eructation and distention. During the week prior to admission, he noted the sudden appearance of a painful red spot on the dorsum of his left foot, which persisted until the day of admission. Following this, he experienced sharp, sticking pain in the large toe of the same foot. This lasted twenty-four hours. The next day he felt a similar pain in the large toe of the right foot. Four days before admission he noted a painful red spot in the palm of the right hand. The day before entrance he felt extremely short of breath and complained of precordial pain and orthopnea.

In his past history the only illness he knew of, besides the rheumatic affections noted above, was pneumonia with empyema in early childhood.

His family history was negative.

Physical Examination: On admission the patient appeared well developed, fairly well nourished, but acutely and chronically ill. He exhibited dyspnea at rest and orthopnea of choice. The head jerked with each systole of the heart. Pallor was marked, but there was no notable tinting of the skin. The conjunctivae were the seat of numerous petechial hemorrhages with white centers. The sclerae were bluish. The pupils were round, regular, and reacted to light and accommodation. In the fundi of both eyes numerous retinal petechiae were seen. The nose was negative for abnormalities. The teeth were in fair condition and the tongue was moist. Few petechiae were visible in the mucous membrane of the hard palate. The veins of the neck were not distended. Markedly accentuated carotid pulsations were visible.

The chest was deformed on the left side due to retraction of the third and fourth ribs and interspaces in the anterior axillary line. There was dullness over the right base, and a leathery rub could be heard on the left side from the angle of the scapula down. It had no relation to respiration and was thought to be pleuropericardial. Many moist râles were audible over the base of the left lung.

The point of maximum intensity of the cardiac impulse was difficult to locate. The impulse was diffuse and was visible in the sixth interspace in the midaxilla, 13.5 cm. to the left of the midsternum. There was a visible systolic retraction in the third and sixth interspaces. A systolic thrill was palpable over the pulmonic area, none over the apex. Slight tenderness was elicited over the precordium. The first sound at the apex was replaced by a murmur which began in mid-diastole and lasted through systole. It was musical in quality. P_2 was greater than A_2 and accentuated. Over the base of the heart a roughened systolic murmur was heard and an early blowing diastolic murmur which was loudest at Erb's point. The ventricular rate was 120 and of regular rhythm. The pulse was of the Corrigan type, rate 120. A pistol-shot sound was heard over the brachials and femorals. The blood pressure was 160/0.

There was moderate distention and tympanites of the abdomen. The liver and spleen were not felt, and there were no signs of fluid. No edema of the extremities, or clubbing of the fingers or toes, was noted. There was an elevated, tender, red node, 1 cm. in diameter, in the center of the palm of the right hand. A similar node which was not tender was seen on the dorsum of the left foot. There were many petechiae in the skin of both the upper and lower extremities.

Laboratory Data: Urinalysis showed a specific gravity of 1020, albumin+, no sugar, no acetone, many white and epithelial cells, numerous red blood cells, and many hyaline and granular casts.

The urine sediment count (Addis) for a 12 hour specimen gave the following results: pH 5.0, specific gravity 1020, albumin+, red blood cells 1,799,900, white and epithelial cells 4,240,500, and casts 3,198,000 (chiefly granular).* The blood Wassermann was negative. The non-protein nitrogen of the blood was 35 mg. per cent, sugar 90 mg. per cent. The hemoglobin was 70 per cent (Dare), the red blood cells numbered 3,828,000, the leucocyte differential count was polymorphonuclear leucocytes 87, lymphocytes 10, and monocytes 3.

Blood cultures, five days and two days ante mortem, yielded a Gram-negative coccus in pure culture.

Electrocardiographic examination five days before death showed sinus tachycardia with arrhythmia and incomplete intraventricular block (partial bundle branch block). (Electrocardiograms taken a year previously showed only changes in the R-T segments and occasional tachycardia.)

Course: On admission the temperature was 104° F, the pulse rate was 110. Subsequently the temperature ranged between 102° and 105° for six days. The pulse range was between 100 and 130. His toxic state grew more profound. Petechiae appeared in showers in the skin of the shoulder, arms, chest and neck. He became stuporous and died on May 31, 1930, six days after admission, with terminal pulmonary edema.

Clinical Diagnosis: Cardiac† (A) *Etiological:* Rheumatic fever, inactive, active? Gram-negative coccus.

(B) *Anatomical:* Enlarged heart, adherent pericardium, mitral stenosis, mitral insufficiency, aortic insufficiency, bacterial endocarditis.

(C) *Physiological:* Sinus tachycardia.

(D) *Functional:* Class III.

Autopsy Findings

There was moderate edema of the lower extremities. Numerous petechial hemorrhages were seen in the conjunctival sacs, buccal mucous membranes and in the skin of the entire body. The peritoneal cavity contained a slight increase in fluid, which was clear and straw-colored. Both pleural cavities contained a slight amount of serosanguineous fluid. The pericardial sac was entirely obliterated by old fibrous adhesions. Where it could be stripped the revealed surface contained numerous petechial hemorrhages.

* Normal values for 12 hour specimens should not exceed: red blood cells, 500,000, white blood cells, 1,000,000, and casts up to 5000.

† Cardiac diagnosis conforms to the nomenclature recommended by the American Heart Association.

The lungs were edematous and hyperemic. The visceral pleurae showed numerous petechial hemorrhages. There were no other changes.

The heart was markedly enlarged and weighed 945 gm. All the chambers were dilated. On section the myocardium appeared to be infiltrated by a moderate number of diffusely scattered, small, white and yellowish white deposits of pin-head size. The myocardium of both ventricles was considerably hypertrophied. The tricuspid valve and its chordae tendineae appeared normal. The pulmonary valve was slightly thickened and showed beginning fusion of the cusps. Small, firm, verrucous vegetations were visible between the anterior and posterior cusps. The mural endocardium in the left auricle above the mitral valve showed several small verrucous vegetations with definite thickening of the entire endocardium. The mitral valve (Fig. 1) was diffusely thickened, rigid, moderately stenosed, and measured 11.5 cm. at the base. Its chordae tendineae were somewhat thickened and shortened. A large, friable, reddish brown vegetation measuring 2.5 cm. by 7 cm. by 3.5 cm. was found attached to the auricular surface of the aortic cusp of the mitral valve. The aortic valve showed thickening and rolling of the edges with fusion of the commissural edges. In addition there was a row of fine verrucous vegetations on the ventricular surface. Beneath the valve the endocardium was markedly sclerosed. Several small creeping vegetations were found on the chordae tendineae of the mitral valve. The aorta and coronary vessels appeared normal.

The spleen appeared markedly enlarged, weighing 690 gm. It was soft and very friable on section. A small, firm infarct was found along the inferior border.

The liver was considerably enlarged, weighing 2600 gm. Its capsule was smooth and intact. On section the markings of chronic passive congestion were visible. The gall-bladder appeared normal.

The gastro-intestinal tract was normal except for the presence of numerous petechial hemorrhages on the serous surface of the intestine.

The kidneys were of normal size and their capsules stripped easily, leaving a smooth surface which showed many small petechial hemorrhages. A small, anemic infarct was found at the superior pole of the left kidney. On cut-section numerous pin-point and linear streaked hemorrhages were seen.

Examination of the other organs showed no notable pathological changes.

Bacterial cultures were made of blood of the inferior vena cava and fragments of the vegetation on the mitral valve.

Microscopic Findings

Heart and Great Vessels: Study of the mitral valves (Figs. 2, 3 and 4) revealed a densely sclerosed valve with moderate cellular infiltration. These cells were chiefly lymphocytes. In places there was evidence of a fresh inflammatory process with areas of edema and surrounding collections of polymorphonuclear leucocytes, histiocytes and a few lymphocytes. In some sections verrucae were seen which were indistinguishable from those seen in rheumatic verrucous endocarditis. On the auricular endocardium, some distance from the vegetation, were found small verrucae composed of fibrin on a proliferated base containing many histiocytes.

Sections of the mitral valve, which included the vegetation, showed the base to be fairly well organized by the deposition of many fibroblasts, which could be seen growing into the thrombotic vegetation. The vegetation itself was composed of deeply staining masses of fibrin, leucocytes and large clumps and colonies of bacteria, which were found to be *Gram-negative when stained by the MacCallum-Goodpasture method.* (A control section was stained by the same method.)

Sections of the aortic and pulmonary valves confirmed the gross diagnosis of chronic valvulitis and acute verrucous endocarditis.

Sections of the myocardium revealed extensive changes throughout. Dense, acellular, fibrous connective tissue was found around the blood vessels. Interstitial connective tissue was increased throughout. In areas, irregular connective tissue scars were seen interrupting muscle bundles and replacing them. The intact muscle was composed of hypertrophied fibers and hypertrophied nuclei. Numerous large and small miliary abscesses were found in the myocardium of both ventricles and the left auricle (Fig. 5). No Aschoff bodies were seen. Bacterial stains of sections through the abscesses were unsatisfactory because of the large amount of pyknotic nuclear material. Occasional areas of necrosis were found, which showed invasion by lymphocytes and proliferation of fibroblasts. Several small branches of the coronary arteries showed purulent thrombi.

Sections of the aorta (Fig. 6) were interesting because of the finding of flame-shaped scars extending from the adventitia into the media, interrupting the elastic lamella. These were considered characteristic of the healed stage of rheumatic aortitis as described by Pappenheimer and Von Glahn.[3] Many of the nutrient arteries showed sclerotic and endarteritic changes.

Sections of the other organs confirmed the gross diagnosis.

Final Pathological Diagnoses: Acute bacterial endocarditis of the mitral valve and its chordae tendineae; chronic valvulitis of the mitral, aortic and pulmonary valves; acute verrucous endocarditis of the mitral, aortic and pulmonary valves, and of the left auricle; healed aortitis (rheumatic); hypertrophy and dilatation of the heart; multiple abscesses of the myocardium; fibrosis of the myocardium; aortic insufficiency, mitral insufficiency; adhesive pericarditis; chronic passive hyperemia of the lungs; pulp hyperplasia of the spleen, infarct of the spleen; acute focal embolic nephritis, anemic infarct of the left kidney; chronic passive hyperemia of liver; petechial hemorrhages of the skin and serous membranes; edema of the feet.

Bacteriological Findings

Blood culture done five days before death yielded a pure growth of a Gram-negative coccus in broth and on blood agar plates. An identical strain was recovered from the heart's blood at autopsy, and from the fresh vegetation taken from the mitral valve. As noted above in the microscopic findings, the sections of the vegetation showed the colonies in it to be Gram-negative. On blood agar plates the colonies were smaller than meningococcus colonies. They were firm, tenaciously adherent to the medium; in broth and salt solution they sedimented spontaneously. After 48 hours the surface of the colonies became corrugated.

The colonies attained the size of 2.5 to 3 mm. They were slightly irregular in outline but had smooth borders. They were somewhat raised, glistening, opaque and colorless. After prolonged growth they could be removed as dried masses.

The organisms grew on the surface of all media, exhibiting constant characteristics. No pigment was formed at 37.5° C, at room temperature, on potato, Loeffler's serum or plain agar media. Culture in daylight and darkness was not accompanied by pigment production. Gelatin was not liquefied.

In peptone water indol was formed. The organisms grew slowly in 2 per cent dextrose agar under anerobic conditions and formed no gas.

The following table indicates the behavior with sugars, inulin and milk:

Filtered maltose	Acid	Gas
Maltose	"	"
Dextrose	"	"
Levulose	"	"
Saccharose	"	"
Lactose	—	—
Dextrine	—	—
Mannite	—	—
Inulin	—	—
Milk	—	—

One rabbit was inoculated intravenously with 1.5 cc. of a 24 hour broth culture, one guinea pig was injected intraperitoneally with 1 cc., another guinea pig was given 2 cc. intraperitoneally with no ill effects, and one mouse was injected intraperitoneally with 0.5 cc. The animals were killed after two weeks and gross and microscopic examination of their tissues showed no pathological changes.

Comment

The portal of entry for the infecting organism in this case is obscure. We are forced to assume that a bacteremia occurred some time prior to the onset of symptoms and that the organisms became implanted on the deformed mitral valve. While it is generally held that pathogenic organisms frequently become implanted on diseased heart valves, it is unusual for non-pathogenic bacteria to do so, or even to become the etiological factors for vegetative endocarditis. However, instances have been recorded of such endocardial lesions associated with other non-pathogenic bacteria.

As noted above, Schultz's case was an infection apparently *sui generis*. Of interest in connection with this type of case is a report by Coulter [4] in 1915 of bacterial vegetative endocarditis due to an unknown Gram-negative micrococcus, and the case recently reported by Dickar [5] due to the *Bacillus acidi lactici*. In both cases reported no preëxisting disease of the heart was found. One can only speculate on the factors which seem to render these organisms pathogenic for man in special circumstances.

SUMMARY

A case of bacterial endocarditis (malignant) caused by the *Micrococcus pharyngis siccus* is presented in a human subject with preexisting valvular disease of rheumatic origin.

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DESCRIPTION OF PLATE

PLATE 62

FIG. 1. Close-up photograph of the mitral valve. Note the verrucae along the margin of the anterior leaflet; also note the mural lesion in the auricle above the large vegetation.

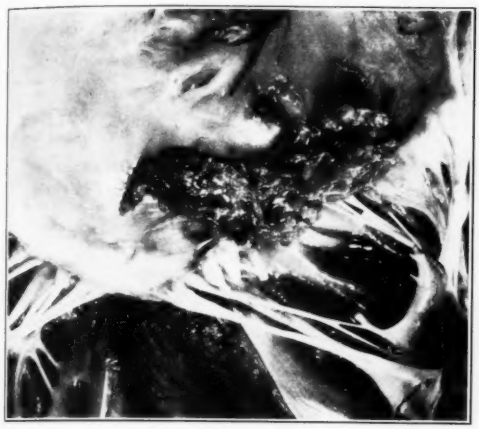
FIG. 2. Low power photomicrograph of a section through the mitral valve and vegetation. × 10.

FIG. 3. Low power photomicrograph of a section through the anterior cusp of mitral valve showing verruca composed of fibrin deposited on base consisting of proliferated histiocytes, lymphocytes and fibroblasts in a hyalinized and sclerosed valve cusp. × 75.

FIG. 4. Photomicrograph to show clumps and colonies of cocci in the vegetation on the mitral valve. × 600.

FIG. 5. Low power photomicrograph of a section of the left ventricle showing abscess formation in the myocardium. × 75.

FIG. 6. Low power photomicrograph of a section of the aorta showing scars in the outer layers of the media, with interruption of the elastica, and slight round cell infiltration. Van Gieson-Weigert elastica-iron hematoxylin. × 75.



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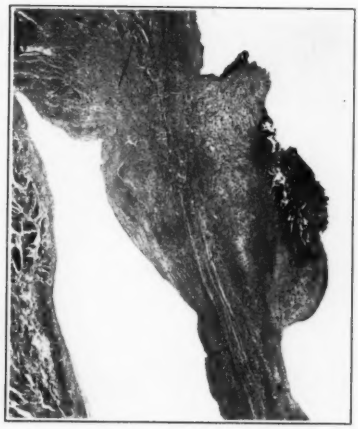

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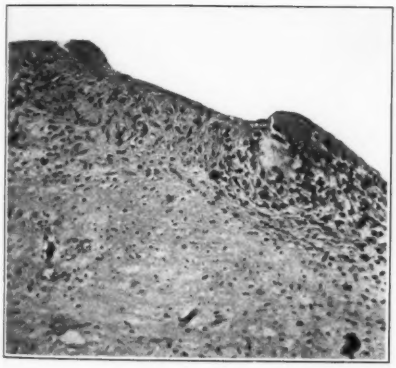

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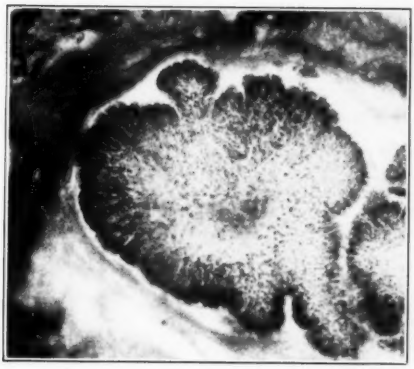

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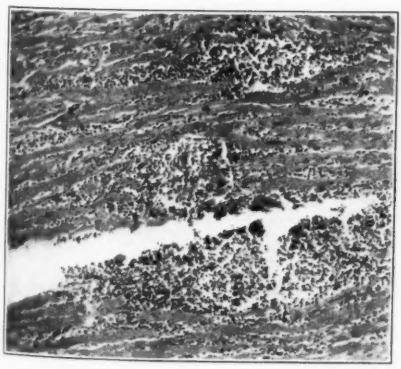

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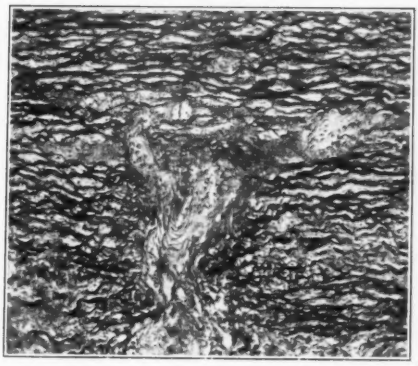

6

Graef, de la Chapelle and Vance

Micrococcus Pharyngis Siccus Endocarditis

EFFECT OF RADIUM EMANATION ON THE HISTOCYTE IN THE LIVER OF THE WHITE RAT *

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The histocytes of the liver lie along the hepatic trabeculae and in the portal spaces. They may be closely attached to the reticular network of the lobule or they may project well into the lumen of the sinusoid. Frequently, in either normal or pathological conditions of the liver they may become detached and migrate through the hepatic parenchyma to the portal spaces, or they may enter the blood stream and pass to other organs of the body. In a normal liver these histocytes are distributed rather irregularly along a sinusoid, but are slightly more abundant in the median and peripheral portions of the lobule. They are ordinarily elongated or spindle-shaped, but when stimulated to activity they assume various forms so that the stellate outline originally employed to describe them is often seen.

Many functions are attributed to these littoral cells. Chief among these, perhaps, is that of defense, for their phagocytic capacity for either organic or inorganic materials is truly enormous. The contribution of these histocytes to pigment metabolism, either by the intracellular digestion of erythrocytes or by engulfing dissolved hemoglobin, is well known. Data concerning the relation of these cells to normal and pathological lipin and carbohydrate metabolism are gradually becoming available.

We shall not attempt to review the extensive literature covering the various pathological conditions in which these littoral cells react, for it is sufficient for our purpose to mention only those related in a measure to this study. Mallory,[1] long ago, described the marked proliferation and the extensive phagocytosis of blood cells in typhoid fever. Similar conditions maintain in malaria, kala-azar, typhus fever, subacute bacterial endocarditis and chronic streptococcal infections.[2] In inflammatory processes these cells desquamate, transform into polyblasts and fibroblasts, and in chronic silica poisoning the formation of connective tissue has been ascribed to them.[3] In

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the repair of small lesions in the liver these cells migrate toward the site of injury and contribute to the formation of a delimiting capsule surrounding the injured zone.[4] Comparable reactions have been displayed by these cells of the liver in the presence of autogenous transplants such as kidney and muscle.[5]

Although the literature on the cytological effects of radium emanations is enormous, we are not aware of recorded studies on the reaction of the histocytes in the liver to such radiant energy. Accordingly this study was undertaken to determine the extent of injury within the immediate field of irradiation, as well as the degree of recovery and the restorative powers of local histocytes more remote from the source of this energy. Only healthy white rats of our colony, ranging in age from 4 to 6 months and in weight from 125 to 175 gm. were used.

Experimental Method

The gold seeds used to introduce the radon into the liver were about 3 mm. long and 0.6 mm. in diameter (outside). The wall of these tiny cylinders, about 0.2 mm. thick, absorbs all but about 0.36 per cent of the beta rays, but permits the passage of 82 per cent of the gamma irradiation.[6] Using aseptic technique, the liver was delivered through a median-line incision and by means of a suitable spinal puncture needle the radon seed was introduced into the hepatic parenchyma to a level about 0.5 cm. below the capsule. Bleeding was slight and easily controlled. Each radon seed contained approximately 1.16 millicurie.

In a series of animals used as controls operation was performed in the same manner, and valueless or inactive gold seeds, but otherwise identical in every respect, were introduced into the corresponding lobe of the liver. The control and the irradiated animals were killed at intervals ranging from six hours to three months. Sections of the liver containing the gold seeds were excised, fixed, and stained with hematoxylin and eosin, Van Gieson's stain and Mallory's stain for connective tissue.

In order to follow the reaction of these cells to the inactive seeds as well as those containing radon, it was desirable to mark them in such a way as to facilitate identity. The extensive phagocytic activity of these cells and the retention, for long periods of time, of engulfed materials provided adequate means for their subsequent

identification. Two intravenous injections of 0.5 cc. of a graphite preparation on successive days, originally described by Drinker and Churchill, was sufficient to distend these cells and make their subsequent identification in histological sections satisfactory (Fig. 1).

Observations

Trauma was, of course, incidental to the introduction of these seeds into the parenchyma of the liver, and slight hemorrhage, together with the formation of an area of necrosed hepatic parenchyma, was unavoidable in all animals. Accordingly the reactions sustained after twenty-four hours in both the control and the irradiated portions of the liver were essentially identical. A zone of compressed, necrosed hepatic cells, blood cells and fibrin surrounded the site of the seed, and scattered graphite from destroyed histocytes was distributed throughout. Any destructive effect of radon on the histocytes of the parenchyma, adjacent to the site of the seed, was certainly not apparent at the end of the first day, for in both the control and the irradiated parenchyma these cells were enlarged several times and projected well into the sinusoids. This apparent stimulation is probably due to the presence of foreign bodies in the form of gold seeds and to the engulfed graphite and the acacia in which the pigment was suspended.

After seventy-two hours, however, striking contrasts in the character and in the extent of the reactions of the parenchyma of the control and of the irradiated livers were observed. The injury induced within the liver containing the inactive seed showed marked recovery, and the degree of restoration at this time was comparable to that observed by Higgins and Murphy at seventy-two hours after the induction of small inflammatory reactions in the liver of rats. The lesion produced by the inactive gold seed was clearly divisible into two zones (Fig. 2). The inner of these consisted of the necrosed material incident to the insertion of the seed and was composed of fibrin, necrotic nuclei, polymorphonuclear leukocytes and fibroblasts. The outer zone, which effectively delimited the inner zone from normal peripheral parenchyma, was composed largely of graphite-laden mononuclear cells. These cells appeared to us to have migrated from their littoral position along the sinusoids to the lesion, and there contributed toward the formation of this cellular wall. Migration to the lesion took place along well defined paths in

portal spaces, for there was evidence to indicate first an accumulation of histocytes in adjacent portal spaces and thence a migration to the lesion. Graphite-laden cells within this wall varied in size and shape. Many of them were spherical or ovoid, whereas many others were attenuated and already exhibited the tendency to their subsequent development.

The effectiveness of the irradiation by the radon seed was clearly seen at the end of the third day, and the induced lesion was in marked contrast to the reaction in the liver of the control animals. The zone of injury was triple the depth of that observed at the end of the first day, and there was no differentiation into an inner and outer zone such as characterized the lesion which developed around the control seed. The injured area, equal in radius to the diameter of the seed, consisted of much fibrin, necrotic material, free graphite and many necrotic histocytes. The region of intense injury continued rather imperceptibly into the peripheral normal parenchyma where vacuolization of the cells and a pale staining reaction indicated injury to hepatic cells. Histocytes immediately peripheral to the region of maximal injury in many instances had been broken down and had given up their pigment granules as a result of the irradiation. In regions more remote from the active seed, beyond the immediate influence of the irradiation, graphite-laden histocytes showed signs of some activity, although much less than that encountered at the same interval in the control animals. In the irradiated livers histocytes did not accumulate in the portal spaces or migrate along paths to the source of the irradiation.

Recovery from the injury induced in the control animals, initiated by the third day, was essentially complete by the third or fourth week. Necrotic tissue incident to the insertion of the seed had been removed and scar tissue remained to indicate the site of the foreign body implant. The lobules in the parenchyma adjacent to the lesion were practically free of graphite-laden histocytes, and new mononuclear cells, probably of local as well as of extraneous origin, had taken their places along the sinusoids. Portal spaces, however, contained extensive accumulations of graphite-containing giant cells, fibroblasts and histocytes, and the scar tissue at the site of the seed implant contained much carbon pigment.

The destructive effect of the radon seed on the liver continued for at least five or six weeks, varying somewhat in the animals examined.

In general the maximal injury was induced some time between the fourth and the sixth week. The radius of the necrotic zone in these livers reached approximately twice the diameter of the gold seed by the fourteenth day and at least triple that extent by the thirty-fifth day (Fig. 3). At this time the transition from the necrotic zone to normal parenchyma was more abrupt than hitherto seen, and one may rightly conclude that the maximal effective destruction had been reached.

During these weeks there was but slight activity among the histocytes, either those closely adjacent to or even more remote from the lesion. There were no indications toward restoration such as occurred in the normal animals. In some of the rats studied at the fourteenth and the twenty-first day a slight migration of graphite-laden cells into adjacent portal spaces had occurred, but these were so infrequent as to merit slight recognition in the total processes of recovery.

The concluding observations, which were made at ten and twelve weeks after the introduction of the radon seeds into the liver, showed rather clearly that active histocytes were engaged in the process of recovery (Fig. 4). The hepatic parenchyma was free of graphite cells except in the portal spaces, and new littoral histocytes were distributed along the sinusoids in characteristic positions. The necrotic zone, although not completely absorbed, was reduced in extent and was completely isolated from the adjacent parenchyma by a wide wall or capsule formed of connective tissue fibers and mononuclear cells heavily studded with graphite. With connective tissue stains the fibers were clearly delineated and the distribution of graphite granules among them served to suggest that a transformation of the histocyte into connective tissue had taken place.

Discussion

In this study of the reaction of the local histocytes in the liver to the radon, as contained in gold seeds, we have not attempted to follow the detailed cytological changes that were induced. We wished to know: (1) whether the littoral histocytes in the liver were any more resistant to radon than the parenchyma cell; (2) when the maximal injury had occurred, and (3) the recovery and the contribution of these cells to the restoration of the injured part. We have

not attempted to follow the reticulum of the hepatic lobule in this study, but it would be interesting to know whether these reticular fibers supporting hepatic parenchyma lose their identity when subjected to radium irradiation, as they do when exposed to the Roentgen-ray.[7] Reticulum is probably a product of these local histocytes. It is common practice to designate Kupffer's cells as reticular cells, and the belief is current that reticulum bears some genetic relation to them. However, the exact origin of this fibrous network from reticular or littoral cells in the liver has never been established. Mallory and Parker [8] derived reticulum of the liver lobule from fibroblasts of the stroma and not from these littoral lining cells. And yet there has been some evidence which would seem to indicate that these local histocytes may transform into fibroblasts. In cultures of adult mammalian connective tissue Maximow [9] showed clearly that this silver-stained fibrillar network arises as the result of a precipitation or a transformation of some colloidal substance under the influence of unknown factors which originate within the cell. Undoubtedly reticulum in the hepatic lobule is precipitated from reticular cells in much the same way.

The reaction of the local histocytes to a foreign body, for in reality the control inactive gold seeds used in this experiment constituted a foreign body, was strikingly identical to that encountered in response to trauma induced in the liver by a small instrument. Those cells actually traumatized by the insertion of the seed were destroyed, and within twenty-four hours polymorphonuclear leukocytes appeared in abundance to remove the necrotic cells and the liberated graphite. During the time of this preliminary neutrophilic reaction local histocytes manifested activity, in that they retracted their processes, buckled into the sinusoids, and often were freed from their reticular attachments. The character of the stimuli inducing these reactions within regions considerably remote from the zone of injury is unknown. It is probably chemical. Many of these graphite-laden histocytes passed either directly to adjacent portal spaces or often directly to the lesion, and well defined paths leading to the necrotic zone were often clearly delineated by the heavily laden, graphite-containing mononuclear cells. Seventy-two hours after operation a rather well defined wall composed largely of these graphite cells had formed around the necrotic zone separating it

from the peripheral normal parenchyma (Fig. 2). This reaction is identical to that seen by Higgins and Murphy in livers of rats seventy-two hours after the induction of slight trauma, and it confirms the observations of Biebl, who studied the reactions of the Kupffer cell to foreign bodies in the form of autogenous transplants such as kidney and muscle. Animals of the control series, which were killed at one week after placing the inactive seed into the liver, presented conditions in which the mononuclear wall was more compact. The peripheral parenchyma was relatively free of graphite cells and a more definitely compact wall separated the necrotic zone from the normal tissue. With acid fuchsin stains the connective tissue in the wall was delineated and graphite granules were abundantly distributed among its fibers. Many of the graphite-laden cells had retained a spherical or slightly ovoid contour, whereas many others were attenuated and resembled typical connective tissue cells. Here, then, were additional data which confirmed earlier opinions that hemophages of the liver may transform into or give rise to connective tissue cells. Following ligation of the major blood vessels to the kidney of the rat, Jordan, Kindred and Paine [10] followed the activity of both fibroblasts and macrophages in the infarcted kidney. These cells had their own particular functions and there was no evidence that macrophages become transformed into fibroblasts, which, on the basis of our knowledge of active macrophages and fibroblasts, one should probably anticipate. It may be, however, that in later stages, as in advanced inflammation, such a transformation of macrophages into fibroblasts might take place. Cultures of organs have shown that large ameboid macrophages proliferate mitotically and finally transform into fibroblasts with an elaboration of the silver-stained, collagenous fibers. Reticular cells of the liver or of the spleen, however, may hardly be regarded as typical macrophages, although they are functionally phagocytic and may become macrophages when stimulated to activity by any foreign material.

When portions of the liver were irradiated by radon, histocytes were as susceptible to injury as any parenchyma or cell of the biliary duct. Within the necrotic zone seventy-two hours after operation, disintegrated or fragmented histocytes and scattered graphite mingled with the necrosed parenchyma and fibrin. There was no attempt at recovery, or organization, such as was seen around the

inactive control seed. In fact, there was no initiation of restorative activity by peripheral histocytes until after the fifth week, when the maximal injury induced by the irradiation had been effected. The necrotic zone increased progressively in extent of its radius until at thirty-five days it was triple the diameter of the seed. At this time the fragmented histocytes were restricted largely to the peripheral portion of the lesion, whereas the medial and central portions consisted practically of fibrin and scattered carbon granules, together with a few mononuclear cells of unknown origin. Polymorphonuclear leukocytes were not present.

Recovery and organization of the lesion, as far as the participation of the histocytes was concerned, commenced some time between the fifth and the sixth week. Up to this time histocytes peripheral to the influence of irradiation had remained essentially inactive. Some retraction had occurred, but the desquamation, so to speak, or the migration so characteristic of these cells surrounding the control inactive seed during the first few days had not taken place. The duration of the destructive influences of the irradiation, if one is to judge by the cellular reaction in these livers, was approximately five to six weeks and is therein comparable to clinical data on the effectiveness of radon. Whether the pathological changes encountered were wholly due to the radon or to subsequent products of decay within these gold seeds is, of course, unknown.

Although the maximal injury was attained at five to six weeks, recovery and organization of the lesion were greatly retarded. There appeared to be prolonged effects, so that cells which normally would have acted quickly to stimuli responded but feebly. Observations made at seven, eight and nine weeks after the onset of irradiation showed only a slightly active histocytic system in the parenchyma surrounding the lesion. Gradually, however, a reaction ensued wherein these littoral cells, still containing the engulfed graphite, proceeded toward the lesion and formed a heavy wall around the now presumably inactive gold seed. In the last of the series of irradiated animals killed at twelve weeks after the insertion of the radon seed (Fig. 4), a reaction was noted, comparable in many respects to that seen in the livers of control animals after three days to one week. Here were large numbers of mononuclear cells laden with graphite, which together with connective tissue fibers formed a

compact wall separating the now inactive seed from the adjacent portions of parenchyma.

Accordingly, at twelve weeks after inserting radon into the hepatic lobes of rats cytological reactions were derived, rather closely resembling those seen at the end of one week in animals which had received the control seed. The maximal injury to the liver by the radon was apparently reached by the fifth or sixth week, and yet outlying histocytes made little if any contribution to recovery until the ninth or tenth week. Factors in this delay are not at once apparent, but we feel that they may be correlated with toxic influences set up by the destructive effects of the radon. Subsequently all histocytes not within the range of the effect of radon pursued a normal trend, in that they desquamated and migrated toward the foreign body and contributed toward the formation of the delimiting wall.

The evidence from this study sustains the opinion that reticular cells of the liver may transform into connective tissue cells. Normally such changes probably do not occur, and we should not suppose that Kupffer cells, while functioning in their normal capacity along the sinusoid, would produce connective tissue. Their function as Kupffer cells in regions more remote from the site of the radon was probably unimpaired during the interval the irradiation was effective, and there were no indications that fibroblasts had been produced along the sinusoids. After, however, the macrophagic function of these cells has passed and their function as a Kupffer cell in its relation to the physiological function of the liver has ceased, then these cells appear to acquire connective tissue potency and may transform into the highly specialized or differentiated fibroblast. Goldzieher and Hornick,[11] in a consideration of the source and the fate of the hyperplastic reticular cells in reticulosis, concluded that especially in the spleen, but also in the liver, definite fibrosis occurred. There was no proliferation of fibroblasts and the conclusion was reached that, under such conditions at least, Kupffer cells may transform into fibroblasts. These conclusions sustain our observations that when reticular cells of the liver no longer function as normal littoral cells, then transformations into the more highly differentiated fibroblast may take place.

Summary

1. A study has been made of the reaction of the histocytes in the liver to radon, as contained in gold radon seeds. Similar but inactive gold seeds were used in a series of control animals to determine the local reaction to such foreign bodies.

2. All local histocytes within the vicinity of the inactive gold seed responded immediately to the foreign body by retracting their processes, desquamating either into the sinusoids or migrating into adjacent parenchyma. Their function as littoral histocytes had apparently ceased and they were now concerned in an attack on the foreign body. Seventy-two hours after insertion of the inactive seed into the parenchyma an effective barrier had been formed around it by these actively migrating histocytes. Subsequently these cells contributed toward the formation of connective tissue.

3. All histocytes in the vicinity of the active radon seed were as susceptible to the emanation as the cells of the parenchyma. The extent of the destruction by the radon increased until the maximal injury was reached about the thirty-fifth day. Histocytes within the zone of injury were destroyed, and those immediately beyond did not manifest signs of restorative activity during this period. After the fifth week, however, a retarded restorative activity was manifested by the histocytes just peripheral to the zone of maximal injury. By the tenth or twelfth week these cells considerably remote from the radon seed, and which either had not been affected or only slightly affected by the radon, migrated rapidly toward the seed and contributed toward the formation of a wall comparable to that seen three days after the implantation of the control seed.

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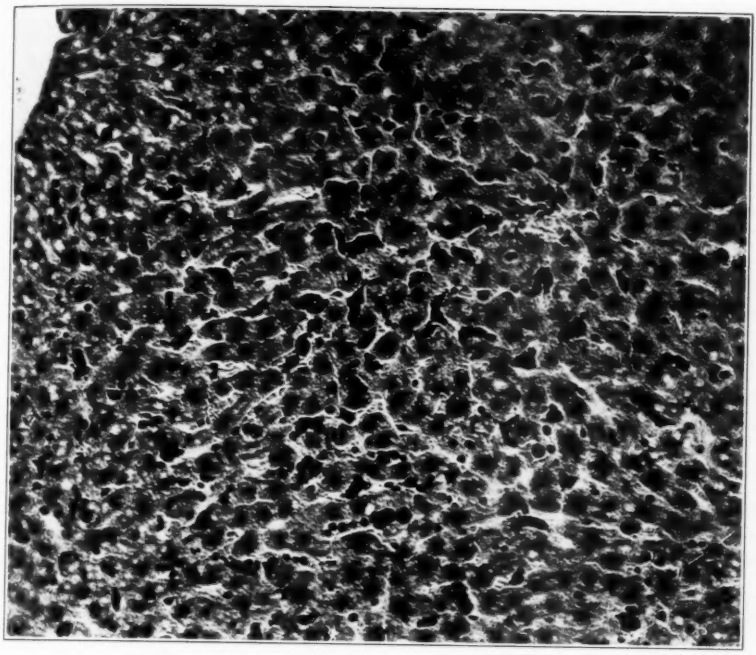
DESCRIPTION OF PLATES

PLATE 63

FIG. 1. Liver of rat, following injection of graphite; littoral histocytes prior to insertion of radon seed are shown.

FIG. 2. Lesions in liver of rat seventy-two hours after insertion of inactive gold seed into hepatic parenchyma. Graphite-laden cells have migrated toward the lesion.



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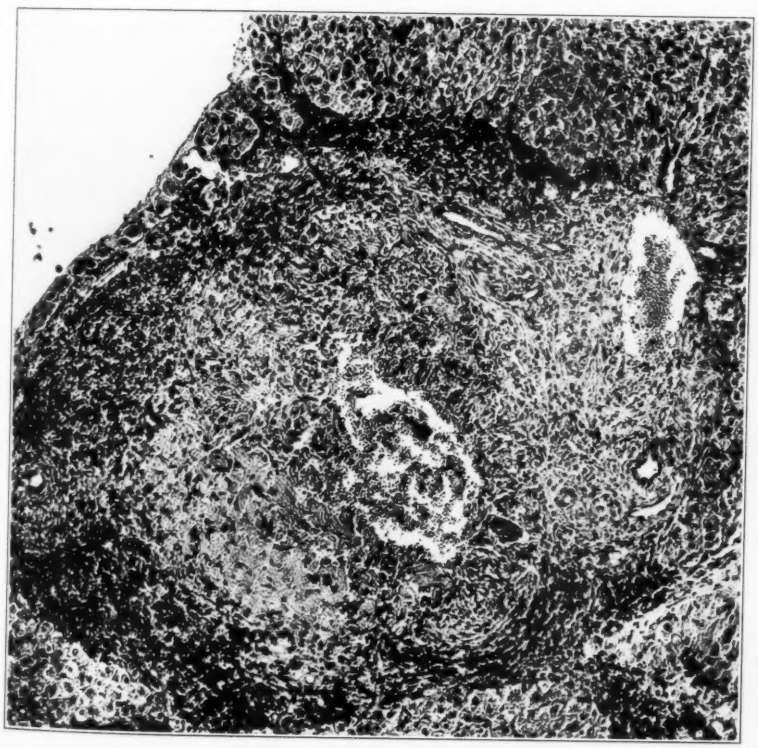

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PLATE 64

FIG. 3. Lesion in liver of rat fourteen days after insertion of active radon seed into hepatic parenchyma.

FIG. 4. Lesion in liver of rat twelve weeks after insertion of active radon seed into hepatic parenchyma.

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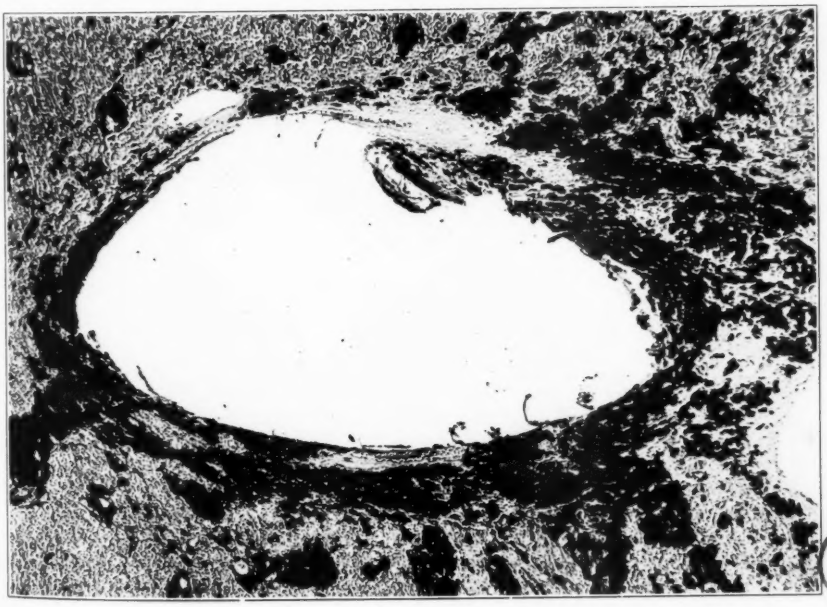

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